Abstract notebook

XIV INTERNATIONAL SYMPOSIUM ON VASOACTIVE PEPTIDES

LAGOA DOS INGLESES, MINAS GERAIS, BRAZIL

The International Symposium on Vasoactive Peptides is a unique forum for graduate students, post-docs, clinicians and other scientists to present and exchange new data and ideas.
The first edition of the symposium was held in Ouro Preto in 1991. Since then, other twelve editions , mostly bi-annual, have been organized in different cities of Minas Gerais.
The XIV International Symposium on Vasoactive Peptides will be held in Nova Lima and will have as one of its main topics the recent advances on the classic and new roles of the renin-angiotensin system in health and disease. New aspects of other peptidic system such as kinins and apelins will be also addressed in the meeting.
The symposium will provide young scientists a venue to expand their knowledge of recent advances in the field, as well as, discuss and receive feedback on their research. The meeting is also designed to foster interactions and constructive networking with peers and established scientists.

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BRAIN AND NEUROSCIENCES

702596 CENTRAL ACTIONS OF ANG-(1-7) ON THE CARDIOVASCULAR RESPONSES OF RATS SUBJECTED TO HYPOVOLEMIC HYPOTENSION: A POSSIBLE ROLE FOR VASOPRESSIN

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Introduction: The central mechanisms involved in the regulation of blood pressure during hemorrhage have not yet been fully clarified. Some brain regions have been studied because they play a vital role in the regulation of body fluids, autonomic and cardiovascular changes, among these regions we can highlight the paraventricular nucleus of the hypothalamus (PVN). Recent studies demonstrate that endogenous peptides of the renin-angiotensin system (RAS) modulate blood pressure at different stages of hemorrhage, indicating that a possible imbalance of these peptides may be involved in changes in blood pressure modulation during hemorrhage. Aim: To characterize the role of endogenous Ang-(1-7) in the PVN in the cardiovascular alterations that occurs during the different phases of hemorrhage. Methods: For this purpose, Wistar rats were admitted to bilateral microinjection of A779 (1 nmol/100nL) and to a hemorrhage protocol in a fixed volume of 24 mL/kg for 20 min, performed simultaneously with the recording of mean arterial pressure (MAP, mmHg) and heart rate (HR, bpm) in freely moving rats. Recorded data were used to assess MAP, HR and autonomic function parameters (analyzed through HR and systolic blood pressure (SBP) variability by spectral analysis). Results: The microinjection of A779 into PVN attenuated the blood pressure (BP) drop induced by hemorrhage (decompensated phase), possibly related to the increase in HR in this period. The blockade of Mas receptor also induced a faster recovery of BP after the end of the hemorrhage (recovery phase). Rats treated with A779 also showed a reduction in mean HR spectral density, RMSSD (variable related to parasympathetic activity), abolition of the increase in LF and HF components that occurs at the end of hemorrhage in the Saline group, in addition to a decrease in BP liability during the onset of bleeding. To evaluate the participation of systemic vasopressin on the effect induced by A779 microinjected into the PVN, rats treated with i.v. administration of V1 receptor antagonist (50µg/mL/kg) were used. The blockade of V1 receptor abolished the effect induced by A779 into PVN in the reduction of magnitude of hemorrhagic hypotension. Conclusion(s): This study suggests that Ang-(1-7) acting on the PVN as a modulator of BP and HR during the decompensated and recovery phases of hemorrhage, possibly by modulating vagal tonus and vasopressin synthesis/release in these phases.

Key words: Hemorrhage, Renin Angiotensin System, Hemorrhagic shock, Neurophysiology, Cardiovascular Physiology and PVN.

730273 **CONTRIBUTIONS OF AT1A RECEPTORS WITHIN SIM1 NEURONS ON NEUROENDOCRINE CONTROL OF ENERGY BALANCE**

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Background: The paraventricular nucleus of the hypothalamus (PVN) regulates sympathetic outflow, release of stress hormones, and signaling of thirst and appetite. Neurons within the PVN robustly express AT1a receptors that mediate angiotensin II (Ang II) effects on cardiovascular function, autonomic balance, food intake and energy expenditure. Lesions of the PVN results in obesity and decreased brown adipose tissue (BAT) thermogenesis, while exaggerated Ang II/AT1a signaling promotes obesity through mechanisms that remain undefined. Therefore, we aimed to investigate the role of AT1a receptors in the PVN neurons on metabolism in C57BL/6 mice fed normal diet. Methods: Young adult male and female conditional knockout mice (AT1a-/-Sim1cre+, n=4) in which AT1a receptors are excluded from Sim1-expressing neurons, and their littermate wild-type mice (AT1a+/+Sim1cre+, n=6) were used in the study. The metabolic data was obtained by confining the mice in the promethion chamber for five consecutive days. Body lean fat composition was measured by NMR, and gene expression of key targets was assessed by real-time qPCR. Results: Compared to the WT mice, AT1a-/-Sim1+ mice exhibit reduced body weight but no difference in adiposity and BAT ratio. Lean mass is decreased in AT1a-/-Sim1+ female compared to WT. Glucose tolerance test was similar between the WT and AT1a-/-Sim1+ mice. Furthermore, AT1a-/-Sim1+ mice exhibit reduced oxygen consumption and CO2 production. Interestingly, despite the respiratory rate and energy balance being similar between the two groups, we find significant reductions in energy expenditure and water consumption in the AT1a-/-Sim1+ mice. Real time PCR showed marked decreases in ß-adrenergic receptors (ß1, ß2, and ß3), and marked increases in UCP1 in the BAT of AT1a-/-Sim1+ mice. Expressions of AT1a and MrgD (ligand for alamandine) were significantly attenuated in the BAT of AT1a-/-Sim1+ mice. In the brainstem, MAS (ligand for Ang-1-7) was decreased but MrgD expression was increased. In contrast, we find that both AT1a and MAS expression were decreased, MrgD was increased, and ß1 expression was decreased in the left ventricle of AT1a-/-Sim1+ mice. Conclusions: AT1a in the PVN plays an important role in regulating energy balance and gene expression of RAS receptors and thermogenic markers. We propose that, to compensate for the lack of AT1a signaling in the PVN, opposite pathways such as reduced MAS signaling in the brainstem and left ventricle, increased UCP1 and decreased ß-adrenergic signaling in the BAT are likely upregulated. Here, we have shown that conditional KO mice for AT1a within Sim1 neurons can potentially alter energy balance when fed a normal chow diet. To fully elucidate the contribution of PVN - AT1a in metabolism we will next investigate how a caloric challenge can modulate energy balance in the AT1a-/-Sim1+ mice (Funding: NIH R01HL149677 and R21AG070188).

Key words: RAS, AT1a receptors, PVN, Sim1 neurons and metabolism

749672 **MRGD DEFICIENCY LEADS TO HYPERACTIVITY AND COMPULSIVITY IN MICE**

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The renin-angiotensin system (RAS) is a pivotal regulator of central nervous system (CNS) homeostasis. Within the CNS, hyperactivation of the angiotensin type 1 receptor (ATR1) is known to induce detrimental effects, including inflammation and cell death. In contrast, the angiotensin type 2 receptor (ATR2), angiotensin type 4 receptor (ATR4), MAS receptor (MasR), and the MAS-related G protein type D (MrgD) receptor trigger neuroprotective responses. Recent studies established connections between the RAS and the development of neurodegenerative and cognitive disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD), dementia, anxiety, and depression. Moreover, proteomics analysis of cardiac tissues in MrgD receptor-deficient mice has revealed the regulation of proteins associated with neurodegenerative processes, indicating that MrgD signaling could play a significant role in the CNS. Our study aimed to assess the behavioral, neurochemical, and post-translational modifications neuroproteomics (PTMomics) in C57Bl6/J (WT) and MrgD knock-out mice (KO) at different ages. For this evaluation, both groups underwent behavioral tests to assess motor, emotional, and cognitive behavior. Subsequently, the mice were euthanized, and regions such as the substantia nigra (SN), striatum (ST), prefrontal cortex, motor cortex, hippocampus (HPC), and amygdala were collected for neurochemical and PTMomics analysis. Surprisingly, the data showed that KO mice exhibited motor hyperactivity and a high degree of compulsivity at all ages evaluated, without anxiety and with no alterations in short- or long-term memory. Additionally, the neurochemical profile of the nigrostriatal and limbic pathways was significantly altered, with an accumulation of Dopamine (DA), Norepinephrine, and Epinephrine in the SN, a decrease in DA in the ST, and an increase in Serotonin in the HPC in KO mice. PTMomics data from the nigrostriatal pathway in KO mice also showed alterations in biological processes related to dopaminergic and glutamatergic synapse, learning, motor activity control, and synaptic vesicle exocytosis. Besides that, there were alterations in glycosylation and abundance of proteins in the N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, such as Syntaxin (Stx1a), Syntaxin-binding protein (Stxbp1) and Complexin 2 (Cplx2), which play a crucial role in anchoring and fusion of vesicles containing neurotransmitters to the presynaptic plasma membrane. Moreover, intracerebroventricular Alamandine treatment reduced compulsivity in rats with an attention-deficit/hyperactivity disorder like phenotype, indicating MrgD receptor's influence. In conclusion, these findings shed light on how the MrgD receptor and Ala impact behavior and neurochemistry, particularly in the nigrostriatal system, with potential relevance to neuropsychiatric disorders involving motor control and impulsivity.

Key words: Renin-Angiotensin System, MrgD receptor, Alamandine, Hyperactivity, Compulsivity, Neurochemistry, PTMomics.

CANCER AND INFLAMMATION

677417 **EVALUATION OF CIRCULATING RENIN-ANGIOTENSIN SYSTEM COMPONENTS IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA: A PILOT STUDY**

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Background: Acute leukemia (AL) is the most common cancer of childhood. Aim: To assess whether there is an association between the blood levels of Renin Angiotensin System (RAS) molecules in children with acute AL and disease presentation and evolution in pediatric patients. Methods: This is a cross-sectional study carried out in a group of pediatric patients with AL. We measured blood levels of Angiotensin II (Ang II) and Angiotensin-(1-7) [Ang-(1-7)] by enzyme immunoassay. The Ang-(1-7)/Ang II ratio was calculated as a parameter of the balance between the alternative and classical axes of the RAS. Results: Eleven patients with AL and 20 healthy controls matched by sex and age were included. Patients with AL had significantly higher levels of both peptides when compared with healthy controls ($p < 0.05$). However, no significant difference was found in the Ang-(1-7)/Ang II ratio between the two groups. A strong and positive correlation was detected between Ang II and Ang-(1-7) levels in patients with AL ($r = 0.853$; $p < 0.0001$). There was no significant difference between the levels of Ang II and Ang-(1-7), as well as the Ang-(1-7)/Ang II ratio, the type of AL and clinical outcomes. Conclusions: Both Ang-(1-7) and Ang II seem to be involved in the physiopathology of AL and other molecules of the RAS could be potentially explored for the development of new therapeutic options for AL.

Key words: Acute leukemia, Renin angiotensin system, Angiotensin II, Angiotensin (1-7).

743134 **THE DES-AGR9-BRADYKININ/B1R AXIS: HEPATIC DAMAGE IN COVID-19**

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Introduction: Patients with SARS-CoV-2 infection often experience liver damage associated with drug-induced therapies and systemic viral action. Dysregulation of the kallikrein-kinin and renin-angiotensin systems has been suggested as a possible mechanism for multi-organ damage. This study aimed to investigate the plasma concentration of biologically active peptides from the kallikrein-kinin system and the expression of the bradykinin 1 receptor (B1R) in the liver of COVID-19 patients. Aim: Our study aims to evaluate whether SARS-CoV-2 infection alters the circulating concentration of the biologically active peptides bradykinin (BK) and des-arg9-BK, as well as the hepatic expression of their proinflammatory axis, B1R. Methods: This study was approved by the local ethical board (CAAE30152620.1.0000.0005). Plasma concentrations of bradykinin and des-arg9-bradykinin were measured in 15 COVID-19 patients diagnosed by RT-qPCR using a liquid chromatography mass spectrometry-based methodology. We selected and prepared the tissues and then evaluated the expression of B1R by immunohistochemistry of post-mortem liver specimens from 27 individuals with COVID-19. All statistical assessments were performed in Prism (GraphPad Software, San Diego, CA). Results: We found a significantly increased ($p < 0.05$) plasma concentration of des-arg9-BK in 18 COVID-19 patients, compared to the 18 volunteers in the healthy control group. Among 15 COVID-19 patients, there was a significantly reduced ($p < 0.0001$) concentration of bradykinin when compared to the 21 healthy individuals in the control group. We also observed a significant increase ($p < 0.0001$) in B1R expression in the liver tissue of COVID-19 patients. In the liver, the mean B1R expression level among this group was 125.9 ± 11.0 a. u., while in the control group, it was 101.6 ± 5.2 a. u. Conclusions: Our data indicate that the des-arg9-BK/B1R axis may be associated with hepatic tissue injury in the pathophysiology of COVID-19 and suggest that it is a mechanism responsible for acute liver dysfunction induced by SARS-CoV-2 virus infection.

Key words: COVID-19, Inflammation, Bradykinin, Des-Arg-Bradykinin

749307 **ANGIOTENSIN II REVERTS THE INHIBITION OF THE ACE, ACE2, AND MAS RECEPTOR EXPRESSION INDUCED BY RECOMBINANT SARS-COV-2 SPIKE PROTEIN IN BRONCHIAL SMOOTH MUSCLE CELLS**

Juliane Stephanie Mendonça Rodrigues, Tarciana Guedes, Victor Gustavo Balera Brito, Sandra Helena Penha de Oliveira.

Background/Introduction: Coronavirus disease 2019 (COVID-19), is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has several important structural proteins, including spike (S) glycoprotein. Spike glycoproteins recognize and bind the surface receptor angiotensin-converting enzyme 2 (ACE2). The renin–angiotensin system (RAS) is a cascade involved in human pathophysiology. In RAS, renin converts angiotensinogen into angiotensin I. The angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) into angiotensin II (Ang II). Ang II binds to receptors AT1 and AT2. ACE2 is an enzyme that catalyzes the cleavage of Ang II into Ang (1-7), and Ang (1-7) binds to the MAS receptor. Aim: This study aimed to investigate whether SARS-CoV-2 spike protein (Sp) induces expression of ACE, ACE2, and MAS receptor (MASr) and produces IFN-gamma and the modulation of Ang II on this response in human bronchial smooth muscle cells (BSMC). Methods: Sp (1 ug, 5 ug, and 10 ug/mL) were used to stimulate BSMC (5x104 cells/well) and the expression and cytokine production were evaluated after 3, 24, and 48h. The treatment of Ang II (100 ng/mL) was used in three different conditions: i) Spike (5 ug/mL) + Ang II (100 ng/mL) (spike protein added together with Ang II (100 ng/mL), ii) Spike (5 ug/mL) + Ang II (100 ng/mL), but the Ang II added 3hs after of the spike protein treatment; iii) Ang II (100 ng/mL) only; iv) no treatment (normal medium culture). The expression of ACE, ACE2, and MASr were evaluated by real-time RT-PCR. The IFN-gamma production was evaluated by ELISA. Results: BSMC expressed constitutively ACE, ACE2, and MASr. The treatment with Sp (5 ug/mL) inhibited ACE expression after 3h and 24h. Ang II was added together with Sp (5 ug/mL) or added after 3h reverting the inhibition of Sp at 3h after the stimulation. At 24h Sp (5 ug/mL) also inhibited ACE expression but only when Ang II was added together with Sp (5 ug/mL) reverting the inhibition. The expression of ACE2 was inhibited by Sp (1 ug/mL) after 3h. At 24h, Sp (5 ug/mL) inhibited the ACE2 expression, and Ang II added at the same time reverted it. Sp (5 ug/mL) did not modify MASr expression, but the addition of Ang II induced an increase of this receptor after 3h. At 24h, Sp (1 and 5 ug/mL) or Ang II alone inhibited the expression of the MASr, but Ang II was able to revert this inhibition induced by Sp. At 48h Sp (5 ug/mL) or Ang II inhibited the expression of the MASr, but only Ang II added at 3h after was able to revert it. The expression of IFN-gamma decreased after stimulation of spike (5 µg/mL) and Ang II, and Ang II was added 3h after Sp reverted it. The IFN-gamma production was increased by Sp (10 µg/mL) after 3 and 24h. Ang II was added at 3h after inhibiting this expression induced by Sp (5 ug/mL). There was no difference among the stimuli after 48h. Conclusion: Sp inhibited ACE, ACE2, and MASr expression and IFN-gamma production, and Ang II seems to modulate it.

Key words: Angiotensin 2, SARS-CoV-2, ACE2, Spike protein

753003 **ALAMANDINE DIMINISHES TUMOR PROGRESSION AND MUSCLE WASTING IN TUMOR BEARING C57BL/6 MICE**

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Introduction: Cancer-associated cachexia is a prevalent complication observed in approximately half of cancer patients, associated with a poorer prognosis and increased mortality rates. The involvement of the Renin Angiotensin System (RAS) in cancer development and muscle physiology related to cachexia has already been established. Previous research has explored the potential of angiotensin-(1-7), a heptapeptide of the RAS, for its anti-tumoral and anticachectic effects. Additionally, our group has previously demonstrated the antiproliferative properties of a newly described heptapeptide, Alamandine. Aim: We sought to evaluate the antitumoral effect of Alamandine in a melanoma model using C57BL/6 mice and investigate its potential anticachectic effect. Methods: Male C57BL/6 mice were injected with a suspension of B16F10 cells into the right flank. The mice were then randomly divided into two groups: the control group (received oral gavage of HPßCD at a dose of 82µg/Kg/day; n=5) and the treatment group (received oral gavage of Alamandine at a dose of 50µg/Kg/day; n=5). Throughout a 14-day period, the mice weight and tumor volume were monitored. At the end of this period, the mice were euthanized, and their tissues were collected for subsequent analysis. Both plasma and melanoma tissue samples were collected to measure the levels of circulating peptides using LC-MS/MS. Additionally, muscle and adipose tissue samples were collected to analyze the cachexia index. Muscle wasting was evaluated through histology and Western Blotting techniques. Results: We observed a significant reduction in tumor mass and volume after a 14-day treatment with Alamandine (50µg/Kg/day). In the melanoma tissue of the Alamandine-treated animals, we found a decreased abundance of ERK1/2 and an increased abundance of ACE2. Moreover, the treated group exhibited lower levels of AngII in the melanoma tissue, along with higher levels of circulating Alamandine and AngI. Furthermore, the body mass of the animals in the treatment group was preserved compared to their initial body mass. This preservation of body mass indicated an anticachectic effect, which was further supported by the inhibition of muscle and fat wasting. The percentage of muscle and fat mass relative to the initial body mass demonstrated the following results: Gastrocnemius muscle: CT 4.03±0.12% vs. Ala 4.82±0.21%; Tibialis anterior muscle: CT 1.16±0.06% vs. Ala 1.65±0.13%; Soleus muscle: CT 0.20±0.01% vs. Ala 0.32±0.025%; Heart muscle: CT 3.67±0.08% vs. Ala 4.55±0.18%; Epididymal fat pad: CT 0.61±0.27% vs. Ala 4.29±1.14%. These findings collectively demonstrate the significant impact of Alamandine treatment on tumor regression, modulation of signaling molecules in melanoma tissue, and its potential to prevent muscle and fat wasting associated with cachexia. Conclusion: Our data reveals an anti-tumoral and anticachectic effect of an oral formulation containing Alamandine in tumor bearing mice.

Key words: cancer, cachexia, alamandine, SRA

749905 **LUNATIN-1: A PROMISING ANTITUMORAL PEPTIDE FROM SCORPION VENOM WICH INDUCES CELL DEATH IN MDA-MB-231 THROUGH CELL MEMBRANE DAMAGE**

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Introduction: Previous studies by our group showed that Lunatin-1, a peptide isolated from the venom of the Peruvian scorpion Hadruroides lunatus, induces apoptosis in the human promyelocytic leukemia cell line HL-60 and causes cell death in human breast metastatic cancer cells MCF-7 and MDA- 231 by unknown mechanisms. Objective: Evaluate the mechanism of cytotoxicity of Lunatin-1 in breast cancer cells. Methodology: Synthetic Lunatin-1 was purified by high performance liquid chromatography (HPLC) and analyzed by mass spectrometry (MALDI-TOF/TOF). The IC50 was determined as 25 μM, which was the concentration used to assess cell viability through propidium iodide (PI) staining and its morphology changes for 1 h using a Cell Imaging Multimode Reader (Cytation). Cell membrane morphology was assessed through Scanning Electron Microscopy (SEM) after 25 μM of Lunatin-1 treatment in different time-points. Results: Treatment of MDA-MB-231 with 25 μM of lunatin-1 induces cell membrane permeability, as evidenced by the presence of cells with positive PI staining from the first minutes of peptide addition ($p<0.05$) remaining up to 1h ($p<0.05$) of treatment when compared to vehicle (0.5% DMSO). It also induces membrane damage evidenced in SEM through reduction of microvilli in cell membrane since the first minute of treatment. Conclusion: Lunatin-1 induces breast cancer cell line death through mechanisms related to membrane damage and can be used as a leading antitumor drug.

Key words: lunatin-1, breast cancer, membrane damage.

754201 **ANGIOTENSIN-(1-7), A POTENTIAL ADJUVANT TO DOXORUBICIN CANCER THERAPY, REDUCES NEPHROTOXICITY BY DECREASING OXIDATIVE STRESS AND FIBROSIS**

Ana Clara Melo, Omeed Rahimi, Patricia Gallagher.

Doxorubicin (Dox) is an effective cancer antibiotic for a variety of solid tumors and blood cancers in both adult and pediatric patients. However, administration of this drug may lead to cumulative toxicity in multiple major organs including heart and kidney. These deleterious tissue effects raise serious concerns regarding the long-term quality of life in patients administered Dox, in particular childhood cancer survivors with decades of life ahead. Dox induces renal oxidative stress, resulting in enhanced inflammation and increased fibrosis, leading to kidney dysfunction. Consequently, there is a need for adjunct therapies, especially in pediatric patients, to reduce Dox-induced renal toxicity and enhance long-term quality-of-life. Angiotensin-(1-7) [Ang-(1-7)] is a heptapeptide hormone of the renin-angiotensin system that activates a specific G-protein-coupled receptor MAS to counteract the hypertensive effects of angiotensin II. Further, the heptapeptide hormone has potent anti-cancer, anti-oxidant, and anti-inflammatory properties. In this study, the potential use of Ang-(1-7) as an adjuvant to protect the kidneys of juvenile rats from acute DOX-induced nephrotoxicity was investigated. Five-week-old male Sprague-Dawley rats (n = 8-10) were randomly assigned to four groups: control (no treatment), Dox, Ang-(1-7) or Dox and Ang-(1-7). Dox (22 mg/kg) was administered by intraperitoneal injection once a week, while rats received continuous infusion of Ang-(1-7) (24 mg/kg/h) by osmotic minipumps. After 6 weeks of treatment, malondialdehyde and 4-hydroxynonenol, markers of lipid peroxidation, were increased greater than 50% in the kidneys of Dox-treated rats; this effect was prevented by co-treatment with Ang-(1-7). The antioxidant enzymes catalase and SOD 1 were increased approximately 2-fold ($p<0.01$) in rats treated with Dox in combination with Ang-(1-7), suggesting that the heptapeptide hormone upregulates oxidative stress defense mechanisms in kidney tissue to reduce reactive oxygen species. Histochemical analysis of Picrosirius red stained kidney sections showed a significant increase in renal cortical fibrosis as compared to tissue sections from control rats ($p<0.05$). The staining of kidney sections from rats treated with the combination of Dox and Ang-(1-7) did not differ significantly from control. Further, administration of the heptapeptide hormone markedly attenuated the Dox-induced increase in the fibrotic markers collagen III and fibronectin ($p<0.05$) suggesting that Ang-(1-7) reduces pathological fibrosis and nephrotoxicity by preventing the enhanced production of oxygen free radicals following DOX treatment. Thus, Ang-(1–7) is a clinically safe heptapeptide hormone with both cardio- and renoprotective properties as well as antineoplastic actions that may serve as an effective adjuvant therapy to improve cancer treatment and mitigate the long-term heart and renal toxicity associated with Dox in pediatric cancer patients.

Key words: cancer, doxorubicin, ang-(1-7), nephrotoxicity

CARDIOVASCULAR PHYSIOLOGY

709873 **SYMPATHETIC NEURAL OVERACTIVITY, ENDOTHELIAL DYSFUNCTION, AORTIC STIFFENING, AND DIMINISHED EXERCISE CAPACITY IN BREAST CANCER SURVIVORS TREATED WITH DOXORUBICIN AND TRASTUZUMAB-BASED CHEMOTHERAPY**

João Eduardo Izaias, RENATA MOLL BERNARDES, Bruna Emy Ono, Diego Faria, Artur De Oliveira Sales, Laura Testa, Camila Motta Venchiarutti Moniz, Jose Maurício Mota, Vera Salemi Cury, Luiz Bortolotto, Maria Claudia Irigoyen, Fernanda Consolim-Colombo, Allan Robson Kluser Sales.

Introduction: Previous evidence demonstrates that breast cancer (BC) survivors who were treated with doxorubicin and trastuzumab-based chemotherapy have an increased risk of developing cardiovascular (CV) disease at least 7 years after completion of cancer treatment. The reasons for the increased CV disease risk are not completely understood, a constellation of physiological changes may contribute. We hypothesized that BC survivors compared with age- and body mass index (BMI), sex -matched healthy controls, exhibit sympathetic neural overactivity, vascular endothelial dysfunction, aortic stiffening, and diminished exercise capacity. Methods: Sixteen women BC survivors, treated with doxorubicin and trastuzumab (age=48±2 years and BMI=28±2 Kg/m2) and fourteen women control subjects (age=46±1 years and BMI=27±1 Kg/m2) were studied. Muscle sympathetic nerve activity (MSNA, Microneurography), endothelium-dependent dilation (brachial artery flow-mediated dilation, BAFMD), aortic stiffness (carotid-femoral pulse wave velocity, CFPWV), blood pressure (BP, Finometer), heart rate (HR, Electrocardiogram), and peak oxygen uptake (VO2peak, Cardiopulmonary exercise testing) were measured. Results: BC survivors were evaluated 8±2 years after the completion of cancer treatment. Burst frequency and burst incidence of MSNA were higher and BAFMD and VO2peak were lower in BC survivors than in controls ($p<0.001$). There were not differences between the groups in CFPWV, BP, and HR ($p>0.05$). MSNA burst frequency and burst incidence were inversely associated with VO2peak or BAFMD ($p<0.05$). Conclusion: Our findings demonstrate that BC survivors have an augmented sympathetic neural activity, endothelial dysfunction, and attenuated exercise capacity, which may help to explain, at least in part, the increased CV risk in this population. Therefore, it is urgent to establish therapeutic strategies (e.g., physical exercise rehabilitation) to restore or alleviate these cardiovascular changes in BC survivors.

Key words: sympathetic activity, endothelium, arterial stiffness, blood pressure, and functional capacity.

730343 **PAZOPANIB INDUCES SYSTEMIC ARTERIAL HYPERTENSION IN PATIENTS WITH METASTATIC CLEAR RENAL CELL CANCER: ROLE OF SYSTEMIC VASCULAR DYSFUNCTION**

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Introduction: Pazopanib is a monoclonal antibody belonging to the class of tyrosine kinase inhibitors (TKIs) used as first-line treatment for patients with metastatic clear renal cell carcinoma (CRCC). This drug increases progression-free survival and overall survival of these patients. Although its results are important, Pazopanib has been associated with cardiovascular toxicity, which leads to systemic arterial hypertension (SAH). However, the pathophysiological mechanisms related to SAH are poorly understood. We hypothesize that oral use of Pazopanib causes macrovascular and microvascular endothelial dysfunction, increases aortic arterial stiffening, and increases peripheral vascular resistance, inducing to a dramatic increase in systemic blood pressure. Methods: Seven participants with CRCC (age: 64±5 years and body mass index: 29.1±8.1 Kg.m2) in first-line treatment with Pazopanib 800 mg/day were studied by 4 weeks. During this follow-up three assessments were carried out (baseline, and two and four weeks of treatment). The evaluations consisted of examinations of endothelium-dependent function by flow-mediated dilation of the brachial artery (FMDBA, Ultrasound-Doppler), and vascular resistance of the brachial artery (RVAB, Ultrasound-Doppler), aortic vascular stiffening by carotid pulse wave velocity -femoral (CFPWV, Applanation Tonometry), reactive hyperemia index (RHI, EndoPAT), heart rate (HR, Electrocardiogram) and casual and 24-hours systolic and diastolic blood pressure (SBP and DBP - Ambulatory Blood Pressure Monitoring). Results: Pazopanib increased CFPWV by 16%, RVAB by 20%, 24 hours-SBP by 13% and 24 hours-DBP by 23% after two weeks of treatment ($p<0.05$ versus baseline for all variables) and these responses remained increased in the fourth week of treatment ($p<0.05$ versus baseline for all variables). Also, Pazopanib reduced FMDBA by 38% in four weeks of treatment ($p<0.05$ versus baseline), but in two there was only a slight reduction. Pazopanib decreases RHI by 14% in four weeks of treatment ($p<0.05$ versus baseline), but not in two. HR did not change during follow-up ($p>0.05$). Conclusion: Our findings revealed in first time that the use of Pazopanib for 4 weeks causes microvascular and macrovascular endothelial dysfunctions, increases RVAB, resulting in SAH. These findings strongly indicate the need for therapeutic strategies that can prevent or mitigate the adverse effects of Pazopanib on the cardiovascular system of patients with CRCC.

Key words: metastatic clear renal cell cancer, tyrosine kinase inhibitors, flow-mediated dilation, pazopanib, hypertension.

746743 **EFFECTS OF LOSARTAN TREATMENT ON CARDIOVASCULAR AND RENAL CHANGES IN RATS WITH EXPERIMENTAL INSULAR STROKE**

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Introduction: Stroke is the second leading cause of death worldwide, with about 12.2 million new cases per year. Surviving patients present several physiological alterations. In humans, damage to the insular cortex (IC) results in a marked increase in baseline heart rate, cardiac molecular changes, arrhythmias and renal function changes. Therefore, investigating strategies that can alleviate the consequences of insular stroke becomes extremely relevant. Previous studies indicate a direct interaction between the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS). Aim: Evaluate the effect of systemic angiotensin II AT1 receptor antagonist losartan on cardiovascular, renal and neurological alterations resulting from experimental insular hemorrhage in rats. Methods: Wistar rats were 1) anesthetized and prepared for unilateral injection of autologous blood (ICH, n = 6) or vehicle (saline 200 nl, Sal IC; n = 6) into the IC, 2) and prepared for recording of cardiovascular variables (mean arterial pressure, MAP; heart rate, HR) (CEUA UFMG 112/2019). Immediately after ICH, separate groups of rats (n = 6 each) were subjected to three days of treatment (single daily i.p. dose) of losartan (los; 10 mg / kg) or vehicle (control; NaCl 0.9%, 0.1 ml / 100g). 3) similar groups with the same treatments underwent ecg and neurological assessments (n=6) using the wire hang test and locomotor activity 4) separate groups were prepared, as above, for the evaluation of the renal function. Results: The ICH group showed an elevated baseline HR (422 ± 10 bpm) compared to the Sal IC group (365 ± 6 P <0.01) without significant changes in baseline mean arterial pressure (MAP). Treatment with los i.p (ICH los) restored HR to baseline levels and decreased baseline blood pressure values, compared with i.p. vehicle-treated (ICH sal) rats (HR: los 358 ± 7 vs vehicle 428 ± 10 bpm P <0.01; MAP: los 99 ± 2 vs vehicle 111 ± 2 mmHg P <0.01). Losartan effects were abolished by A-779, an angiotensin-(1-7) Mas receptor antagonist. Molecular assays showed increased Mas receptor expression in the animals treated with los. The ECG analysis showed ectopies in the ICH group, but not in the Sal IC and ICH los groups. No neurological changes were observed. Preliminary data shows an increase in levels of proteinuria, marker of renal injury, and urinary Gamma-glutamyltransferase (Gamma-GT) activity, specific marker of renal tubular damage, in ICH sal (n=6) that was not observed in ICH los (n=6). Conclusion: Present data suggests that AT1 receptor blockade may attenuate the cardiac and renal alterations post-insular stroke. In addition, the reduction in baseline blood pressure may offer additional cardiovascular protection. Part of these effects may involve activation of Mas receptors. The present study clearly suggests that immediate treatment with losartan can minimize post-stroke cardiovascular and renal risk. Support: FAPEMIG APQ-01128-21; CNPq.

Key words: stroke, insular cortex, cardiovascular changes, renal changes, losartan.

745503 **AT2 RECEPTOR INACTIVATION IS ASSOCIATED WITH SENESCENCE AND FIBROSIS IN THE HEART OF AGED MICE**.

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Background: The heart is subject to different pathophysiological stimuli, which can lead to the development of cardiovascular diseases. Aging corresponds to one of the risk factors for these diseases, with the activation of inflammatory cascades and protein complexes called inflammasomes, in addition to the synthesis and release of cytokines. Also, there is the production of reactive oxygen species and activation of senescence pathways, which lead to structural and functional changes in the heart. One of the systems that acts directly on cardiac function and morphology is the renin-angiotensin system (RAS). Its main peptide effector is Ang II, which, when binding to AT1 and AT2 receptors, triggers antagonistic responses in most cases. Thus, hypertrophic, and inflammatory responses are associated with AT1 and cardioprotective responses with AT2. Aim: The hypothesis of the present study is that the AT2 receptor exerts cardioprotective effects in aging and that its suppression triggers the activation of inflammatory processes, with the activation of inflammasomes, which contributes to the inflammatory phenotype associated with aging. Methods: All animal procedures of this study were approved by the Committee on Ethics in the Use of Animals at the Institute of Biomedical Sciences of University of São Paulo (CEUA nº: 1836280519). To date, twenty-eight male young (4-5 months) and old (18 months) wild-type (WT) or AT2 receptor-inactivated (KO-AT2) male mice have been used to analyze changes resulting from aging: marker expression of p53, p21 and p16 senescence by immunoblotting, morphological alterations such as fibrosis, by picrosirius red staining, and cardiac trophism (HW/TL: heart weight/tibial length ratio). Statistical analysis was performed using two-way ANOVA for comparison between groups. Results: During aging, we observed a reduction in survival of old KO-AT2 mice, as well as a significant increase in cardiac trophism and increased tissue fibrosis and senescence marker p53 and p21 (vs old WT). So far, no alterations were seen regarding the production of reactive oxygen species, but a reduction in the expression of the antioxidant enzymes SOD2 and catalase was observed, which may be the cause of redox imbalance in KOAT2 mice. Conclusion: The AT2 receptor seems to contribute to mitigate the pathological processes involved in aging, however, more experiments are needed to confirm or refute our hypothesis.

Key words: aging, heart, RAS

750459 **ANGIOTENSIN CONVERTING ENZYME 2 KNOCKOUT MICE ARE RESISTANT TO HIGH FAT DIET-INDUCED OBESITY**

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Introduction: Angiotensin converting enzyme 2 (ACE2) is considered as an endogenous counterregulator of the classical renin-angiotensin system (RAS) for decreasing the levels of angiotensin II (AngII), through the angiotensin type 1 receptor (AT1), and increasing Ang-(1-7). Those actions confer ACE2 a protective role in cardiovascular diseases (Zhong et al., 2010; Rabelo et al., 2016; Crackower et al., 2022). This enzyme participates in tryptophan uptake in the gut (Hashimoto et al., 2012; Singer et al., 2012), in homeostasis is widely demonstrated in several metabolic studies (Osterreicher et al., 2009; Silva et al., 2013; Nunes-Souza et al., 2016) and, was recently demonstrated to be the receptor for the entry of the SARS-CoV-2 virus into cells, leading to the emergence of COVID-19 (Jackson et al., 2022), presenting pleiotropic actions. Aims: Our aim was to investigate the metabolic effect of ACE2 deletion sin young adults and elderly mice under high calorie intake condition. Methods: Male C57Bl/6 (WT) and ACE2-deficient (ACE2-/y) mice were analyzed at the age of 6 and 12 months, fed for 20-22 weeks either the standard diet (SD, 10% kcal from fat) or high fat diet (HFD, D12451, 45% kcal from fat). ACE2-/y mice on C57BL/6 genetic background were bred and housed in the Max-Delbrück-Center for Molecular Medicine. The following analyzes were performed: glucose tolerance and insulin sensitivity tests; activity, energy expenditure, food and water consumption; body composition; lipolysis in vivo and in vitro; systemic biochemical analyses; histological and quantitative Real-Time PCR analysis in white adipose tissue. All experiments were approved by the “Landesamt fur Gesundheit und Soziales” (LAGeSo; Berlin). Results: Under SD, ACE2-/y showed lower body weight and fat depots, improved glucose tolerance, enhanced insulin sensitivity, higher adiponectin and lower leptin levels compared to WT. This difference was even more pronounced after HFD in 6-month-old mice, but, interestingly, was blunted at the age of 12 months. 6-month-old ACE2-/y mice presented a decrease in adipocyte diameter even under HFD condition, which was a reflection of the upregulation of lipid metabolism in white adipose tissue, through increased lipolysis and expression of genes involved in lipid regulation, such as hormone-sensitive lipase and adrenergic ß3 receptor. Under HFD, both food intake and total energy expenditure were decreased in 6-month-old ACE2-/y mice, accompanied by an increase in liquid intake, compared to WT mice, fed either SD or HFD. Conclusion: ACE2 deletion interferes with glucose, lipid, and body weight homeostasis. ACE2-/y mice are less susceptible to HFD-induced obesity in age-dependent manner.

Key words: ACE2-/y mice, High fat diet-induced obesity, Lipid metabolism, Glucose metabolism.

750499 **ANGIOTENSIN II-AT1 PATHWAY CONTRIBUTES TO HIGH FAT DIET-INDUCED CARDIOMETABOLIC DISORDERS IN RATS WITH METABOLIC SYNDROME**

Valéria Nunes De SOUZA, Rosa Ariel Bustillo Rivas, Lavínia Beatriz Hermínio da Silva, Jaime Dativo de Medeiros, Luiza Antas Rabelo, Glória I. B. Pinto Duarte.

Introduction: Metabolic Syndrome (MetS) is a multifactorial disease characterized by the cluster of several cardiovascular and metabolic disorders, such as obesity, diabetes, hypertension, dyslipidemia, insulin resistance (Alberti, et al., 2009). Impairment in several signaling pathways correlates with the worsening of the MetS, including the renin-angiotensin aldosterone system. Aims: Our aim was to investigate the effects of AT1 receptor antagonism with losartan on cardiometabolic alterations in rats with dietary metabolic syndrome. Methods: 8-week-old male Wistar rats were randomized into: 1- Control group (CT), which received standard rodent diet; 2- Obese group (HFD), which received a high fat diet (58.4% kcal from fat) for 16 weeks; 3- Control+Losartan Group (CT+LOS), which received standard rodent diet and losartan (15mg.Kg-1/day-gavage); and 4- Obese+Losartan Group (HFD+LOS), which received a high fat diet for 16 weeks and losartan (15mg.Kg-1/day-gavage). Losartan was administered during the final 8 weeks of the dietary intervention. Metabolic (food and water consumption, lipid and glycemic parameters) and cardiac parameters (cardiomyocyte size; lipid and collagen deposition; REDOX analysis; and gene expression) were evaluated. Approved by UFPE Ethics Committee for the Use of Animals (Process: 0012/2019). Results: HFD group demonstrated body weight gain and higher adiposity index compared to the CT group, as well as insulin resistance and glucose intolerance. Pharmacological intervention in the HFD+LOS group improved insulin sensitivity and glucose tolerance. Heart weight was reduced in treated groups (CT+LOS; HFD+LOS) compared to untreated groups (CT; HFD). Cardiac lipids were not different between the groups. However, there was an increase in collagen in the HFD group compared to CT. Interestingly, the HFD+LOS group showed a significant decrease in both interstitial and perivascular collagen deposition compared to HFD. The cardiac REDOX status, measured by the lipid peroxidation and the enzymatic activity of superoxide dismutase, catalase and arginase did not differ between the groups. However, there was a significant increase in nitrite levels in the HFD group compared to CT, which normalized after treatment with losartan (HFD+LOS). The relative gene expression for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), AT1 receptor and angiotensin converting enzyme (ACE) was reduced in the HFD group compared to CT. However, losartan treatment did not alter gene expressions. Conclusion: In summary, the chronic consumption of a high fat diet induced cardiometabolic disorders which characterize MetS in rats and such injuries seem to be mediated by the action of angiotensin II via the AT1 receptor.

Key words: High fat diet, Losartan, Cardiometabolic disorders, Metabolic Syndrome

748217 **ALAMANDINE ATTENUATES OXIDATIVE STRESS IN THE RIGHT CAROTID FOLLOWING TRANSVERSE AORTIC CONSTRICTION IN MICE**

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Introduction. It is known that pressure overload can lead to vascular remodeling. High tension levels promote vascular alterations characterized by inflammation, fibrosis and vessel's structural changes. New components from the renin-angiotensin system (RAS) such as alamandine, can protect the vessel from these deleterious effects. Aim. Our objective was to evaluate in right carotid, the effect of alamandine in vascular remodeling and expression of oxidative stress related substances, induced by transverse aortic constriction (TAC). Methods. Male C57BL/6 mice were distributed in Sham, TAC and TAC treated with alamandine (TAC+ALA) groups. Three days before the surgery the animal's treatment started and continued for fourteen days. Alamandine was administrated orally by gavage in dose of 30 µg/kg/day. After two weeks of TAC, animals were euthanized. The right and left carotids arteries were collected and embedded in OCT. For morphometric analysis H&E were performed. To analyze collagen deposition, we did Picrosirius Red staining. Expression of the proteins and substances involved in oxidative stress was evaluated by immunofluorescence assays and using DAF/DHE probes, respectively. Statistical analysis was performed using One Way ANOVA followed by Newman-Keuls post-hoc test. Results. TAC induced hypertrophic and positive right-carotid remodeling and fibrosis. Alamandine treatment prevented the production of reactive oxygen species and the depletion of nitric oxide. Alamandine treatment also prevented the expression increasement of NRF2 in right carotids and the rise of nitrotyrosine Conclusion. Our results demonstrate that alamandine attenuates the pathophysiological stress in right carotids of animals subjected to TAC, demonstrating its antioxidant properties in response to pressure overload.

Key words: Alamandine, right carotid, transverse aortic constriction, oxidative stress, renin-angiotensin system.

748258 **TIME-DEPENDENT MODULATION OF CARDIOMYOCYTE CONTRACTILITY BY ALAMANDINE**

André Luis Lima Monteiro, Sergio Scalzo, Marcos Eliezeck Dos Santos Inacio, Mário de Morais e Silva, Bruno de Lima Sanches, Anderson Kenedy Santos, KATYANA KALINE SILVA FERREIRA, Robson Augusto Souza dos Santos, Rodrigo Antônio Peliciari Garcia, Stêfany Bruno de Assis Cau, Silvia Guatimosim.

Introduction: Alamandine (ALA), a Renin-angiotensin-system (RAS) cardioprotective peptide was described as a positive modulator of cardiomyocyte (CM) contractility from a model of hypertensive disease. Whether this modulation is susceptible to temporal variations is yet to be described, even though the circadian variation is an important pattern observed in the cardiovascular system, and by members RAS. Aim: To determine the effect of day-night cycle on ALA-induced contractile effects in CMs. If so, to investigate the signaling pathways mediating these effects. Methods: Hearts from 10-14 weeks mice were collected at four timepoints (ZT): ZT2 and 8 (light phase), ZT14 and 20 (dark phase), considering ZT0 as the time in which lights are turned on and ZT12 as lights go off in the animal facility (CEUA/UFMG: 236-2019). For each ZT, we evaluated CMs contraction in response to an ALA concentration-response curve. Then, we evaluated the signaling pathway with antagonists, and inhibitors of nitric oxide (NO) production. Statistics: Non-linear regression, One and two-way Anova, with post-hoc Turkey. Data shown as mean±S.E.M. Results: At ZT2, ALA increased CMs fractional shortening (FS) in a concentration-dependent manner. At ZT14, however, ALA promoted an opposite effect by reducing CMs FS. On the other hand, at ZTs 8 and 20 there was no significant contractile effect of ALA in CMs. Therefore, we demonstrate, at first hand, that ALA modulates contractile function of CMs, exerting opposite effects depending on the time of the day. Based on the EC50% for ALA, as well as the ZTs when it is possible to observe its effect, we used the concentration of 100 nmol/L at ZTs 2 and 14 for the following experiments. Pre-incubation of cells with D-Pro7 (1µM, MrgD receptor antagonist), A779 (1µM, Mas antagonist) or Losartan (1µM, AT1 antagonist) prevented the increase in FS promoted by ALA at ZT2. Conversely, at ZT14 only D-Pro7 abolished the ALA induced reduction in FS. Our results obtained at the light phase suggest the participation of MrgD, Mas and AT1 receptors on ALA effect, while at the dark phase only MrgD is involved. Considering that NO is a key downstream molecule in the ALA pathway, pre-incubation of CMs with L-NAME (10µM - NO synthase inhibitor) prevented the increase in FS promoted by ALA at ZT2. At ZT14, however, L-NAME did not affect ALA-induced decrease in FS. Given these findings, we assessed NO production by CMs in response to ALA. At ZT2, ALA induced an increase in NO production, while at ZT14, changes to NO levels were not detected. Conclusion: Taken together, measurements of contractility and NO production support the conclusion the ALA positive modulatory effect is dependent of NO, while the negative modulatory effect is independent. Our results confirm the importance of understanding how modulation of ALA changes according to the time of the day, and how it affects CM physiology leading to more efficient treatments against cardiac diseases.

Key words: Alamandine, cardiomyocytes, contractility, nitric oxide, day-night cycle, MrgD, AT1

749530 **PROSPECTING BIOACTIVE PEPTIDES RELATED TO THE RENIN ANGIOTENSIN SYSTEM**

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Introduction: The renin angiotensin system (RAS) has undergone significant changes in recent decades. This more complex perspective, unlike the classic unilinear system, led to the group's hypothesis that the RAS may have other vasoactive peptide precursor proteins other than just angiotensinogen. Objective: Develop an algorithm capable of facilitating the prospecting of new peptides chemically similar to angiotensin I. Method: A search for proteins with the best potential was carried out through databases extracted from Uniprot (https://www.uniprot.org/) and Pubmed (https://pubmed.ncbi.nlm.nih.gov/), databases provide this information precisely to the amino acid sequences of proteins for study. The group of proteins chosen was Serpins, as they are from the same family as angiotensinogen. Decapeptide fragments of these proteins were generated and the degree of homology of these peptides with Angiotensin I was assessed. The amino acid alphabet simplification methodology was used, based on the BLOSUM50 substitution matrix. This matrix was computed in the C++ programming language, which allowed the development of a classifier to estimate the homology distance between any two peptide sequences. The method proposed in this case replicates any peptide sequence and seeks to slide an amino acid window present in Angiotensin I over the selected peptides, assigning a score based on the similarity measure obtained from the substitution matrix. The Angiotensin window was defined with a fixed number of amino acids that were aligned from their N-terminal ends and shifted one amino acid at a time until the entire chain was evaluated. This path was responsible for generating an estimate of the distance of Angiotensin I to the group of amino acids belonging to the evaluated peptide, and, at the end of the analysis, a score was computed that made it possible to establish a ranking of similarity between the additional peptides of proteins from the Serpins group and the Angiotensin EU. Result: The ongoing project has already generated a matrix that predicts potential peptides with a high degree of similarity that will be subjected to synthesis and testing. Conclusion: We develop an algorithm that can assign a similarity score between peptides using the BLOSUM50 matrix as basis, enabling the search for possible bioactive peptides related to RAS peptides.

Key words: Renin-Angiotensin System; Angiotensin Receptors; biotechnology algorithm; angiotensin 1

749564 **ELASTASE-2 EXPRESSION IN HUMAN ABDOMINAL AORTIC ANEURYSMS: A NOVEL FINDING IN DISEASE PATHOGENESIS**

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Introduction: The abdominal aortic aneurysm (AAA) is a pathological dilation of the abdominal aorta that can lead to death as a cause of the rupture and bleeding. The AAA main risk factors are being old, men, and having smoking habits. Other risk factors can include hypertension, hyperlipidemia, and family history. This disease has no pharmacological treatment other than repair surgery. The Renin-Angiotensin System (RAS) regulates the cardiovascular system and has been implicated in the pathogenesis of AAA. Elastase-2 (ELA-2) is an enzyme that generates angiotensin-II (Ang II) in arteries and contributes to resistance arteries and vascular remodeling in animal models. Our group demonstrated that Elastase-2 Knockout (ELA-2KO) mice ELA-2KO mice might be less susceptible to develop an aorta dilation in AAA induced by Angiotensin II (Ang II) infusion in mice models. Therefore, Elastase-2 is a candidate factor that contributes to the formation and/or development of AAA. We sought to investigate the expression of Elastase-2 in the AAA human aorta. Methods: Aortic tissue from AAA patients (n=22) was collected during conventional AAA repair surgery, while control aortas were obtained from organ donors (n=10). Clinical data were also collected from both patient groups. Protein expression and RNA quantification for Elastase-2 codify gene CELA2a was analyzed by Western Blotting and qPCR. This project was approved by the Ethic Committee (CAAE: 82879518.6.0000.5440). Results: Our AAA cohort consisted mostly males (77.27% AAA vs. 60% Control, p=0.4), smokers or ex-smokers (90.91% AAA vs. 30% Control, p=0.001) and most of them had hypertension (81.82% AAA vs. 50% Control, p=0.09). Analysis of mRNA expression by qPCR in the AAA group (n=14) showed that CELA2a was upregulated (p=0.005) compared with the control group (n=9). Elastase-2 (CELA2a) protein expression by western blotting was upregulated (p=0.03) in the AAA group (n=14) compared with the control group (n=7). Conclusion: Our data show, for the first time, that Elastase-2 expression in the human aorta, and, is up-regulated in AAA human patients. Our data are showing that Elastase-2 contributes to the development of the aneurysm and aorta dilation, similar to what our research group previously showed in ELA-2 KO mice models. Supported by: FAPESP (grants n. 2017/21539-6; 2018/23718-8, 2022/02162-7), CAPES, FAEPA."

Key words: abdominal aortic aneurysm, elastase 2, angiotensin II, renin-agiotensin system, human sample, aortic tissue

749567 **THE EXPRESSION OF TYPE-1 ANGIOTENSIN II RECEPTOR SUBTYPES IS INVOLVED IN CONTRACTILITY IN A MODEL OF ABDOMINAL AORTIC ANEURYSM IN ELASTASE 2 KNOCKOUT MICE**

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Introduction: Elastase-2 (ELA-2) is an enzyme implicated in the local formation of angiotensin II (Ang II) in vascular beds. Increased local synthesis of Ang II contributes to abnormal morphologic and functional phenotype in arteries, leading to vascular remodeling and aortic aneurism. Our group has reported that ELA-2 knockout (ELA-2KO) mice were less susceptible to developing pathologic aorta dilation as a response to chronic Ang II infusion. In rodents, Ang II activates, among others, two subtypes of receptors: AT1a and AT1b. We are testing the hypothesis that susceptibility to aortic dilation and distensibility, as a hallmark of abdominal aortic aneurysm (AAA), is mediated by increased expression of Ang II receptors AT1a and AT1b leading to mechanical impairments in the aorta. Methods - Male wild-type (C57BL/6J, WT) and ELA-2KO (CELA-2aTm1Bdr) mice were treated with either saline or Ang II (1500 ng/kg/min) for twenty-eight days by osmotic pumps infusion. Following the Ethics Committee for animal experimentation (CEUA: 131/2019) all protocols were divided into four groups: WT treated with saline (WT+SAL, n=5), (ELA-2KO treated with saline (ELA-2KO + SAL, n=5), WT treated with Ang II (WT + Ang II, n=5), and ELA-2KO treated with Ang II (ELA-2KO + Ang II, n=5). The RNAm gene expression of Renin-Angiotensin System elements and Smooth muscle alpha-2 (ACTA 2) and Smooth muscle myosin heavy chain (MYH11) were measured by real-time quantitative PCR (RT-PCR) in the aorta tissue. . Functional reactivity by phenylephrine was assessed in isolated aortic rings in the Mulvany . Data was expressed as a mean and standard mean error and analyzed with ANOVA or Kruskal Wallis tests. Results - Ang II treatment increased AT1b gene expression in the aortic from Ela-2KO + Ang II group in comparison to WT + Ang II group. (p=0.0039). The AT1a receptor gene expression was similar in both groups that received Ang II. To analyze contractility function, the Phenylephrine-induced contractility was verified in isolated aortic rings. The contractility was similar after Ang II infusion in WT mice [117,8 ± 0,6% (WT+SAL) versus 107,8 ± 11% (WT + Ang II)], and in Ela-2KO isolated aortic rings mice [63,8 ± 8,1 % (ELA-2KO + SAL) versus 88,14 ± 8,5 % (ELA-2KO + Ang II)]. The maximal response induced by Phenylephrine was basally lower in Ela-2KO + SAL compared WT+SAL. (p=0.0004) The Ang II treatment similar or not change maximal response induced by Phenylephrine in both strain. To analyze if contractility could be responsible for the difference in contractily observed, the gene expression to ACTA2 and MYH11 were analyzed and they were higher in ELA-2KO + Ang II compared to WT+ Ang II Group. (Myh11 p=0.0007, ACTA2 p=0.0019). Conclusion - ELA-2KO mice are less susceptible to abdominal aortic aneurism development induced by Ang II assessed by reduced muscular degeneration and contractility. Increased AT1b receptor gene expression could be a potential mechanism for it.

Key words: Elastase-2, AT1Rb, ACTA-2, MYH11

749806 **EXPLORING THE RELATIONSHIP BETWEEN GUT MICROBIOTA AND THE RENIN-ANGIOTENSIN SYSTEM: UNVEILING THE IMPACT OF ANTIBIOTIC-INDUCED DYSBIOSIS IN MICE CARDIAC PROTEOME**

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INTRODUCTION: The renin-angiotensin system (RAS) is an important modulator of the cardiovascular system. It is composed by the classical and protective axes, whose deregulation can induce cardiac hypertrophy, inflammation, hypertension and metabolic dysfunction. In specific cardiomyopathies, such as diabetic cardiomyopathy, Angiotensin II (Ang II) levels are increased, which can cause cardiac tissue remodeling and consequent loss of contractile function and, eventually, progression to heart failure. Many studies have demonstrated an interaction between gut microbiota and cardiometabolic pathologies, and the potential critical link in this interaction may be a new functional axis named renin-angiotensin system – gut microbiota axis. Gut microbiota and their metabolites may influence host homeostasis, triggering or contributing to the initiation of pathological processes, but these mechanisms are still unknown. The hypothesis of this study is that gut microbiota regulates RAS and ultimately the cardiovascular system. OBJECTIVE: To evaluate the potential association between gut microbiota and RAS in cardiac homeostasis. METODOLOGY: Dysbiosis was induced in male C57Bl6/j mice by a single oral dose of streptomycin together with ampicillin at 1 g/L in drinking water for 14 days. Plasma peptides were extracted and measured by target peptidomics using UPLC coupled to a triple quadrupole mass spectrometer using the Multiple Reaction Monitoring mode. Cardiac tissue was processed by shotgun proteomics, phosphoproteomics and N-linked glycoproteomics, and later analyzed by LC-MS\MS using Data-Dependent Acquisition mode. The oral glucose and insulin tolerance test was also performed after antibiotic therapy. Fecal microbiota was analyzed by 16S RNA sequencing. RESULTS: Dysbiosis led to an imbalance in RAS plasma peptides highlighting the increase in Ang II and reduction in Ang-(1-7). In animals with gut dysbiosis, it was identified by 16S RNA sequencing a significant decrease in the gut bacterial diversity together with increase of the phylum Bacteroidetes and decrease of Firmicutes. The observed impairment in RAS and gut microbiota are in line with a pro-inflammatory status in dysbiosis mice when compared with normobiosis mice. Through cardiac proteome profiling, it was seen a strong enrichment of signaling pathways related to diabetic cardiomyopathy pathway. In addition, phosphorylation of proteins related to hypertrophic cardiomyopathy signaling were observed. Finally, some proteins regarding the receptor interaction of extracellular matrix pathway were found glycosylated, reinforcing the negative impact of the gut microbiota on the cardiovascular system and metabolism. In addition, a decrease of glucose capture in dysbiosis mice 24 days after antibiotic therapy. CONCLUSION: This study indicates that antibiotic-induced dysbiosis has indeed a deleterious effect in the cardiac proteome which may have a negative impact in the progression of cardiac diseases.

Key words: Gut microbiota, diabetic cardiomyopathy, proteomics, renin-angiotensin system

750181 **TAKOTSUBO SYNDROME AND ANGIOTENSIN-(1-7) ORAL TREATMENT: A PHARMACOLOGICAL AND MOLECULAR ASSESSMENT**

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Introduction: Takotsubo cardiomyopathy (TC) is a cardiovascular condition typically precipitated by stress-inducing events that elevate catecholamine levels, including norepinephrine, leading to excessive stimulation of cardiac tissue, culminating in the development in severa cardiac changes. In light of this situation, ongoing research strives to uncover therapies that can diminish the occurrence of TC or to minimize its deleterious effects. Angiotensin-(1-7) [Ang-(1-7)] is an important component of the renin-angiotensin system (RAS). Previous investigations on Ang-(1-7) showed its favorable effects in the protection of different systems and organs, including the heart, where it has been shown to decrease the release of norepinephrine, regulate cell growth and reduce adverse cardiac remodeling. Objective: Hence, this research aims to establish a mouse model of TC to evaluate the potential beneficial effects of Ang-(1-7) treatment. Methodology: We utilized female C57Bl6/J mice with 8-12 weeks. The animals were divided into two experimental groups, the first (MCT) received an intraperitoneal (IP) dose of isoproterenol (ISO) 300 mg/Kg in a single dose to induce TC, the other group (SL) received an IP dose of saline 0.9% and was used as a control. After establishing the TC model, four experimental groups were used, as follows: (ISO-A) received daily oral treatment with Ang-(1-7) included in cyclodextrin (CD) (105 ug/Kg) from the day -1 administration (IP) of isoproterenol ISO, (ISO-C) received daily oral treatment with CD (60 ug/Kg) on day -1 of ISO, (SL-A) received daily oral treatment with Ang-(1-7) included in cyclodextrin (CD) (105 ug/Kg) from day -1 of IP administration of saline, and (SL-C) received daily oral treatment of CD (60ug/Kg) on day - 1 of IP saline administration. The study was approved by the Committee of Ethics in Animal Use (CEUA) of the UFMG, protocol number #223/2023. In order to evaluate cardiac inflammation, we quantified the presence of inflammatory cells in Hematoxylin and eosin-stained slides. Cardiac hypertrophy was assessed by calculating the heart weight to tibia size ratio (HW/TL). Results: Treatment with ISO(--) induced cardiac hypertrophy, validating the cardiomyopathy (TC) model. There was a significant increase in the inflammatory index in the ISO group compared to the control group that received IP saline. Regarding cardiac hypertrophy, defined by HW/TL ratio, a significant difference was observed between the ISO-A (treated with Angiotensin-(1-7)) and ISO-C (treated with cyclodextrin) groups, indicating a reduction in hypertrophy in the group treated with Ang -(1-7). Conclusion: These preliminary findings support the hypothesis that Ang-(1-7) has a cardioprotective effect in the TC model. The next steps will focus on elucidating the mechanisms of action of Ang-(1-7) in TC pathology and exploring its additional beneficial effects. Financial support: CNPq, Capes and Fapemig.

Key words: Takotsubo Syndrome, Takotsubo cardiomyopathy, Angiotensin-(1-7) [Ang-(1-7)], Cardioprotective effect, Hypertrophy.

748622 **PRELIMINARY DATA ON THE EFFECT OF CANG-(1–7) PEPTIDE ADMINISTRATION IN BSC40 CELLS INFECTED WITH THE VACCINIA VIRUS**

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Introduction: The Renin-Angiotensin System (RAS) regulates essential functions in the human body, such as water and electrolyte homeostasis and blood pressure control. The heptapeptide angiotensin 1-7 (Ang 1-7) is an essential system component. Ang 1-7 binds to the MAS receptor, coupled to the G protein, and generally exerts biological effects opposite to Ang II, such as vasodilation, anti-hypertension, anti-proliferation, and anti-inflammation. Therefore, this peptide is helpful in the development of therapeutic agents capable of neutralizing the harmful effects of Ang II in many diseases. However, Ang-(1-7) has impaired in vivo efficacy due to its rapid proteolytic degradation. To overcome this problem, the cyclization of this peptide was proposed with the enzymatic introduction of a thioether ring linking amino acid residues 4 and 7, thus forming a lanthionine. The resulting cyclized Ang-(1–7) cAng-(1–7) is an analog with greatly improved therapeutic potential, resistance to angiotensin-converting enzyme, and significantly increased stability. Aim: We propose to evaluate the effect of cAng-(1–7) on cells contaminated by Vaccinia virus (VACV) in a pre-test assay. Methods: BSC40 cells were maintained and cultured in Eagle's minimal medium (MEM) supplemented with 10% FBS, 100 µg/ml streptomycin, 100 IU/ml penicillin potassium, and 250 ng/ml of amphotericin B, kept in culture bottles and incubated at 37ºC, in an atmosphere of 5% CO2 for subsequent viral infection. For the assay, BSC40 cells at a concentration of 6.0 x 105 cells per well, suspended in MEM medium, were seeded in a 6-well plate and incubated for 24 hours under the same conditions described previously. After this, two wells containing cells were treated with 200 µL of cAng-(1–7) at a 1.0 x 10-6 M; the peptide was previously diluted in PBS. In the other two wells, 200 µL of the MEM medium was added for control. Then, the plate was incubated in an oven for six hours under the same conditions as previously described. After treatment with the peptide, 100 µL of MEM medium containing 200 PFU/mL of VACV/ml were added to the wells. After this procedure, the plate remained incubated in an oven for approximately 48 hours until the characteristic ECP appeared. The plate was then stained for subsequent counting and calculating the UFC Title. Results: The two control wells that were infected and did not receive treatment with cAng-(1–7) presented an average titer of 6,104 PFU/mL, while the wells that received the treatment presented an average titer of 6,103 PFU/mL. Conclusion: The results presented in this study suggest that the cAng1-7 peptide has potential in vitro antiviral activity against VACV. This potential in the preliminary test serves as a basis for future experiments.

Key words: Renin-Angiotensin System, cAng-(1–7), Vaccínia vírus.

751521 **ANGIOTENSIN-(1-7) MODULATES THE CARDIOVASCULAR EFFECTS OF ENDOTHELIN-1 DIFFERENTLY IN NORMOTENSIVE AND HYPERTENSIVE RATS**

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Introduction: Endothelin-1 (ET-1) is involved in cardiovascular pathologies, such as atherosclerosis, coronary artery disease, heart failure, and systemic and pulmonary hypertension. Previous studies have shown that Angiotensin-(1-7) [Ang-(1-7)] attenuates the pro-inflammatory effects induced by ET-1 in human microvascular endothelial cells and that there is a possible interaction between the Mas and ETB receptors. Aim: To evaluate the influence of the Ang-(1-7) on the cardiovascular effects of ET-1 in normotensive and hypertensive rats. Methods: Male normotensive (Wistar) and spontaneously hypertensive (SHR) rats (12-16 weeks of age) were used. Blood pressure was recorded in awake rats 24 hours after catheterization of the femoral artery and vein. ET-1 (0.3 nmol) was administered before and after the infusion of Ang-(1-7) (7 pmol/min.). Coronary reactivity was assessed using the isolated heart preparation (Langendorff technique). The hearts were perfused with either: ET-1 (10 -10 nmol/L); Ang- (1-7) (10 -9 nmol/L); ET-1 + Ang-(1-7); or A-779 (2. 10 -9 nmol/L) + ET-1 + Ang-(1-7). Aortic reactivity was evaluated in isolated aorta rings. ET-1 (10 -8 mol/L) was administered in the presence or absence of Ang-(1-7) (10 -7 mol/L) or Ang-(1-7) + A-779 (10 -6mol/L). Results: Ang-(1-7) inhibited the increase of blood pressure evoked by ET-1 in normotensive, but not in SHR. ET-1 promoted an initial coronary vasodilation followed by important vasoconstriction. In SHR hearts, ET-1 promoted only coronary vasoconstriction. Ang-(1-7) potentiated ET-1-induced vasoconstriction in normotensive rats, but significantly attenuated the vasoconstrictor effect induced by ET-1 in SHR. The A-779 inhibited the vasodilation observed in Ang-(1-7)+ET-1 treated hearts and attenuated the vasoconstriction potentiation induced by Ang-(1-7) in Wistar hearts. In SHR, A-779 inhibited the attenuating effect of Ang-(1-7) on ET-1-induced vasoconstriction. ET-1 induced an important vasoconstriction in isolated aorta from normotensive and hypertensive rats. Ang-(1-7) potentiated the vasoconstrictor effect of ET-1 in preserved endothelium aortas in normotensive animals but attenuated this effect in hypertensive vessels. A-779 potentiated the aortic constrictor effect of ET-1 in normotensive and inhibited the attenuating effect of Ang-(1-7) in hypertensive animals. Conclusion: These results show that Ang-(1-7) modulates the effects of ET-1 on blood pressure and vascular reactivity differently in normotensive and hypertensive animals. Funding: CAPES, CNPq, FAPEG.
Key words: angiotensin-(1-7, endothelin-1, hypertension

751529 **TREATMENT WITH COMPOUND 21 IN HYPERTENSIVE PREGNANT RATS ATTENUATES CARDIOVASCULAR DYSFUNCTION IN ADULT OFFSPRING**

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Introduction: Hypertension is the main risk factor for the development of other cardiovascular diseases. Increasing evidence suggests that cardiovascular disorders can be “programmed” in utero by adverse stimuli during pregnancy. On the other hand, some exposures to beneficial factors during pregnancy may prevent or mitigate diseases in the progeny. Previous studies have shown that AT2 receptor activation promotes beneficial effects on the cardiovascular system. Therefore, we hypothesize that the treatment of the pregnant spontaneous hypertensive rats (SHRs) with Compound 21, an AT2 receptor agonist, would be beneficial on cardiovascular phenotypes expressed in adult offspring. Objective: To evaluate whether treatment with C21 during the gestation of SHR may attenuate the development of cardiovascular dysfunctions in adult offspring. Methods: SHR were treated with C21 (0.3 mg/kg/day) or saline via an osmotic pump throughout the gestational period (CEUA/UFG 039/2017). The systolic blood pressure (SBP) of the offspring was measured weekly by tail plethysmography from the 6º to 16º weeks of age. The cardiac function (echocardiography), baroreflex, and chemoreflex activity were performed in 16-week-old offspring. Then, the animals were euthanized for evaluation of left ventricular function after ischemia and coronary reactivity in an isolated heart preparation (Langendorff technique) and aortic vascular reactivity in the organ bath system. Samples of the left ventricle were collected for morphological and molecular analyses. Results: SBP of the SHR C21 was lower than the control group and showed greater baroreflex sensitivity (SHR -0.3530 ± 0.06 vs. SHR C21 -0.6594 ± 0.10 ?bpm/?mmHg, P<0.05). Echocardiographic parameters were not different between groups. The time of ischemia-reperfusion arrhythmia was significantly reduced in the C21 group (87.60 ± 34.13 vs. 5.66 ± 4.91 seconds in SHR C21, P<0.05). Coronary vasodilation in response to bradykinin was improved in the treated group (-2.87 ± 1.25 vs. - 12.96 ± 3.28 % in SHR C21, P<0.05). Aortic vascular reactivity was not different between groups. Maternal treatment with C21 reduced cardiomyocyte area (396.9 ± 6.33 vs. 320.5 ± 6.44 µm 2 in SHR C21, P<0.05) and interstitial (15.93 ± 0.85 vs. 8.92 ± 0.49 % in SHR C21, P<0.05) and perivascular fibrosis (1.93 ± 0.14 vs. 1.18 ± 0.13 µm 2 in SHR C21, P <0.05) in left ventricles of offspring. NFAT expression was lower in the C21 group (41,65 ± 3,49 vs. 27,01 ± 2,72 au in SHR C21, P<0,05). Conclusion: These results demonstrate that treatment of spontaneously hypertensive rats during pregnancy with C21 can attenuate the development of hypertension and promote cardioprotective effects in the offspring. These data suggest that AT2 receptor activation during pregnancy may be a potential therapeutic strategy for the prevention of cardiovascular disorders in offspring. Funding: CAPES, CNPq, FAPEG.

Key words: C21, hypertension, cardiovascular

753525 **THE NEW MAS RECEPTOR AGONIST, CGEN-856S, ACTIVATES NITRIC OXIDE SIGNALING IN CARDIOMYOCYTES**

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Introduction: Knowing the cardioprotective effects of angiotensin-(1-7) axis, a new agonist of the receptor Mas have been described: CGEN-856S. The cardioprotective effects of this peptide in vivo were confirmed. Goal: To investigate the effects of CGEN-856S peptide in the production and modulation of the nitric oxide pathway in cardiomyocytes and compare them with those promoted by Ang-(1-7). Methods: In this work we used a combination of molecular biology, confocal microscopy and a model of genetically modified mice with deletion of MAS receptor. The nitric oxide production was evaluated with a nitric oxide sensitive fluorescent probe. We also assessed the cardioprotective effects of CGEN-856S against Angiotensin II in a model of neonatal cardiomyocytes. (CEUA: 138/2018). Results: Cardiomyocytes treated with CGEN-856S showed a significant increase in nitric oxide production. This response was lost in cardiomyocytes from MAS knockout mice. Using western blot, we observed a significant increase in phosphorylation of AKT in the Serine 473 residue after acute treatment with CGEN-856S. We also tested the actions of CGEN-856S against Ang II induced hypertrophic effects. Neonatal cardiomyocytes treated with Ang II for 36 hours showed a significant increase in cell area, increased translocation of GRK5. All these effects were prevented by concomitant treatment with CGEN-856S. When A779 (Mas receptor antagonist) or L-NAME (an inhibitor of nitric oxide synthases) was added to cells treated with Ang II and CGEN-856S, results similar to those found in cells incubated with Ang II alone were seen. Conclusion: In cardiac myocytes CGEN-856S peptide increases nitric oxide and is capable of preventing Ang II hypertrophic effects through the production of NO in a way dependent of receptor Mas activation. These results indicate that CGEN-856S acts very similarly to Ang-(1-7) in cardiac myocytes.

Key words: CGEN-856S, Nitric oxide, Cardiomyocytes

753834 **FEMALE RATS EXPRESSING AN ANGIOTENSIN-(1-7)-PRODUCING FUSION PROTEIN (TG7371) PRESENTS A HYPOTENSIVE PHENOTYPE**

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The renin-angiotensin system (RAS) plays a crucial role in cardiovascular control and fluid balance. Angiotensin (Ang) II/ AT1 receptor axis induces pressor, pro-inflammatory and pro-fibrotic actions, contrasting to the effects induced by Ang-(1-7)/MAS receptor axis, which elicits vasodilation, inhibition of cell proliferation, and attenuation of inflammation and fibrosis. Transgenic animals are important tools to evaluate the effect of chronic alteration in components of the RAS. Recently, we described a new transgenic rat that overexpress a fusion protein that releases Ang-(1-7) in tissues, the TG7371. Here, we conducted a comparative analysis of cardiovascular parameters between female and male rats from this transgenic line. Arterial pressure (AP) was assessed by pletismography or direct measurement through arterial catheter connected to BIOPAC System. Estrous cycle was determined in 15 consecutive days by vaginal smear. Baroreflex bradycardia was evaluated by the heart rate (HR) changes elicited by AP alterations induced by bolus injection of phenylephrine. Cardiac autonomic tonus was evaluated by selective blockade of β-adrenergic and muscarinic receptors. First, we evaluated systolic arterial pressure (SAP) through tail pletismography throughout the estrous cycle, showing that the SAP of both TG7371 and Sprague-Dawley (control) is not significantly different among the different phases. However, SAP of female TG7371 (109±3.1 mmHg, n=10) was significantly lower than the SAP of female SD rats (126±2.4 mmHg, n=12). Similar results were observed using direct measurements of AP with BIOPAC System. HR of female TG737 (341±14.5 beats/min, n=23), on the other hand was not significantly different from female SD (330±6.5 beats/min, n=20). Lower AP was also observed in male TG7371 rats (105±2.0 mmHg n=20 vs 121±0.8 mmHg in SD, n=15), corroborating previous study. Next, we evaluated through Mass spectrometry plasma levels of Ang peptides. TG7371 presented similar circulating levels of Ang-(1-7), Ang II, Ang I, Ala-Ang II or Alamandine than SD control, both male or females rats. Interestingly, female TG7371 presented higher Ang-(1-7) levels (228?25 pg/ ml, n=4) than male TG7371 (98,5?15 pg/ml, n=4). Interestingly baroreflex sensitivity was higher in females TG7371 (1.12±0.05 ms/mmHg, n=10) compared to male TG7371 (0.80±0.04 ms/mmHg, n=8). These findings show that long-term overexpression of Ang-(1-7)-producing protein reduces baseline blood pressure in both male and female TG7371 rats. The hypotensive role of Ang-(1-7) is reinforced by these results, especially considering the normotensive background of the animals. Future studies will evaluate the tissue levels of the RAS peptides and the mechanisms involved in the control of AP in these transgenic rats. Funding Support: Grants from CAPES, CNPQ, FAPEMIG, and INCT NANOBIOFAR.

Key words: Renin-angiotensin system, Angiotensin-(1-7), Female, Transgenic animals, Hypotensive phenotype

754790 **TREATMENT WITH EMPAGLIFLOZIN IMPROVES CARDIAC FUNCTION AND SCAR AREA IN INFARCTED ANIMALS ASSOCIATED WITH INCREASED BAROREFLEX SENSITIVITY AND ATTENUATION OF SERCA2 AND P-PLN EXPRESSION**

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Background: Several clinical and experimental studies have demonstrated improvement in cardiac function in infarcted patients and animals treated with empagliflozin, as well as improved outcomes in patients with heart failure with reduced ejection fraction or preserved ejection fraction. Hypothesis: Treatment with empagliflozin for 14 days (10 mg/kg) is able to promote improvement in cardiac function and reduce post-infarction collagen deposition, secondary to improved baroreflex sensitivity. Methods: Wistar rats were divided into 3 groups: CTL – control (n=10), AMI – acute myocardial infarction (n=10) and AMI+EMPA – acute myocardial infarction + Empagliflozin (n=10). The animals in the infarcted groups were anesthetized and submitted to thoracotomy and ligature of the left coronary artery. The animals in the AMI+EMPA group received 10 mg/kg of empagliflozin daily, through oral gavage, for 14 days. After this period, all animals underwent echocardiographic examination, followed by cannulation of the femoral vein and artery, to evaluate hemodynamic parameters and baroreflex activity. After euthanasia, histological evaluation of the scar area (total collagen area) in the left ventricle of the animals was carried out and the expression of SERCA2 and total and phosphorylated phospholamban in the ventricular tissue was evaluated by western blot. The results were evaluated for normality. Results: In the echocardiographic evaluation, an improvement in diastolic function indexes was observed in the AMI+EMPA group in relation to the AMI and CTL groups: E'/A' Ratio (1.65 ± 0.30 vs 0.58 ± 0.11 vs 0. 98 ± 0.59), E/E' ratio: (16.24 ± 7.38 vs 28.08 ± 6.3 vs 25.05 ± 4.8) and in systolic function indexes in the AMI+EMPA group in relation to the AMI group: EF (50.69% ± 14.5 vs 30.14% ± 9.7 vs 30.14% ± 9.7) and FAC (38.46% ± 7.14 vs 23.94 ± 5 ,7). A reduction in systolic and diastolic blood pressure was observed in the AMI+EMPA (124.4 ± 9.10 / 86.98 ± 3.54 mmHg) and AMI (127.3 ± 6.13 / 87.46 ± 2. 76 mmHg) When compared to the CTL group (136.4 ± 5.9 / 92.95 ± 5.70 mmHg). In the evaluation of baroreflex sensitivity, an improvement in tachy and bradycardic indexes was noted in the treated animals: tachy index (4.95 ± 0.37 bpm/mmHg vs 3.11 ± 0.48 bpm/mmHg) and brady index (1. 54 ± 0.23 bpm/mmHg vs 0.698 ± 0.10 bpm/mmHg). In relation to the total area of collagen in the left ventricle, an increase was noted in the AMI group (8.53% ± 3.13) in relation to the CTL group (0.50% ± 0.17) and its reduction in the AMI+EMPA group (3.06% ± 1.6) in relation to the AMI group. An increase in SERCA2 expression in ventricular tissue was noted in the AMI group (1.61 ± 0.5) in relation to the CTL group (1.00 ± 0.1) and its normalization in the AMI+EMPA group (1.02 ± 0.2). The same behavior was observed in relation to phosphorylated phospholamban (0.54 ±0.3 vs 1.00 ± 0.01 vs 0.90 ± 0.5). Conclusion: Treatment with Empagliflozin in infarcted rats was able to improve the systolic and diastolic function of the left ventricle, as well as attenuate the expression of intracellular calcium handling proteins and reduce collagen deposition in ventricular tissue after myocardial infarction, associated with improved performance of arterial baroreceptors.

Key words: empagliflozin, infarction, SERCA2

756207 **INVESTIGATION OF THE RENIN-ANGIOTENSIN SYSTEM AND INFLAMMATORY MEDIATORS IN THE PATHOPHYSIOLOGY OF ABDOMINAL AORTIC ANEURYSM IN HUMANS: A BIOCHEMICAL APPROACH**

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Background: Abdominal aortic aneurysm (AAA) is a progressive degenerative disease characterized by a chronic inflammatory process, resulting from the gradual weakening of the aortic wall, which can lead to rupture and death. The renin-angiotensin system (RAS) plays a role in numerous physiological processes in the cardiovascular and renal systems. It is also involved in the pathophysiology of vascular diseases, being correlated with the pathogenesis and progression of AAA. **Aim**: We sought to investigate how the renin-angiotensin system could modify the inflammatory mediators in the aorta of AAA humans. **Methods**: Aortas samples were collected from a) patients who underwent open AAA correction (AAA group) at the Hospital das Clínicas de Ribeirão Preto (HCRP-USP), b) patients without AAA (non-AAA group) who were diagnosed with brain death and underwent organ procurement surgery at the same hospital. The aortic tissues were used for ex-vivo experiments and histological analyses. To assess the involvement of RAS and its relationship with inflammatory mediators, ex-vivo experiments were conducted where an aortic fragment was incubated in a culture medium in the absence and presence of RAS agonists and inhibitors, as angiotensin (Ang I), Ang II, Ang 1-7, captopril, chymostatin losartan, PD123319, A779. Subsequently, inflammatory mediators such as interleukins (IL-1, IL-6, IL-8, and IL-10) secreted into the culture medium in the presence of RAS inhibitors were analyzed by ELISA. **Results**: The histological findings indicate morphological differences with decreased expression of smooth muscle cell markers in the AAA group vs. non-AAA group. IL-1 is up-regulated after Ang I treatment ($p=0.02$) and downregulated after AngI+Captopril treatment ($p=0.045$) in the AAA group vs. non-AAA. Treatment with Captopril, Losartan, PD123319, Ang1-7, A779, and Ang1-7+A779 did not change IL-1 secretion. Also, IL-6- is not regulated by RAS as all treatments did not change it. IL-8 is up-regulated after Ang I ($p=0.015$), AngI+Captopril ($p=0.045$), Ang I+chymostatin ($p=0.019$), AngII+Captopril ($p=0.0078$), Ang II+Losartan ($p=0.0033$), Ang 1-7 ($p=0.03$), and A779 ($p=0.05$) in the AAA group vs non-AAA. IL-8 levels no change in the presence of PD123319+AngII. IL-10 was significantly higher in the AAA group compared to the non-AAA group ($p=0.008$). IL-10 is up-regulated after treatment with AngI+Captopril ($p=0.01$), Ang I+chymostatin ($p=0.01$), Ang 1-7 ($p=0.0022$), A779 ($p=0.0058$), Ang 1-7+A779 ($p=0.008$) in the AAA group vs non-AAA. The aorta ex-vivo experiments demonstrated interactions at various targets RAS, exhibiting increased tissue secretion of inflammatory mediators such as IL-1, IL-8, and IL-10 in response to RAS inhibitors. **Conclusion:** Together, our data showed the involvement and importance of RAS components changing and influencing aorta local inflammatory mediators in AAA pathophysiology.

Keywords: Abdominal aortic aneurysm; Renin-angiotensin system; Vascular smooth muscle cell culture; Aorta; Ex-vivo culture; Interleukins; Human tissue donors

749069 **EFFECTS OF RILMENIDINE ON CARDIOVASCULAR PARAMETERS IN RATS WITH EXPERIMENTAL HEMORRHAGIC INSULAR STROKE: CONTRIBUTION OF AT1 AND MAS RECEPTORS**

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Introduction: The insular cortex is involved in modulating the cardiovascular autonomic control. Stroke in this area results in enhanced sympathetic activity, sustained tachycardia, cardiac arrhythmias, and often patient's premature death. Recently, we have developed a model of experimental hemorrhagic stroke at the insular cortex (ICH) of rats that mimics the post-insular stroke changes observed in humans. Previous evidence indicates that peptides of renin-angiotensin system (RAS) can modulate the effects of antihypertensive drugs with central action. Rilmenidine is an antihypertensive drug with central action widely prescribed in the clinic. This drug acts by inhibiting the sympathetic tone in the rostral ventrolateral medulla, where AT1 and Mas receptors play a significative role in autonomic effects. Aim: Firstly, we evaluated whether the use of systemic rilmenidine (ril) could attenuate the ICH-associated cardiovascular effects in rats. Neurological parameters were also assessed. Methods: Wistar rats (CEUA UFMG 109/2022) (n = 28) underwent stereotaxic injections (vehicle or autologous blood) into the right insular cortex; treated with vehicle or ril (1 or 10µg/kg, 3 days, i.p); then: 1) anxiety/compulsive-like behavior, 2) locomotor activity, and 3) muscle endurance. On day 4, heart rate (HR) and blood pressure (BP) were recorded. Results: HCI resulted in marked tachycardia (control 323 ± 5 bpm vs 449 ± 15 after hemorrhage; $P<0.01$) that was not observed in ril-treated HCI rats (after ril 1µg/kg 335 ± 8 bpm; $P<0.01$; after ril 10µg/kg 296 ± 6 bpm; $P<0.01$). Additionally, ril 10µg/kg treatment reduced baseline blood pressure values (control treatment 109 ± 0.5 mmHg vs 101 ± 3 after rilmenidine10µg/kg; $P<0.01$). No neurological parameters were affected after ICH or with ril treatment. Conclusion: Our findings revealed that the centrally acting antihypertensive ril was able to attenuate the tachycardia resulting from ICH. Additionally, from a translational point of view, the reduction in baseline BP produced with the highest dose of ril (10µg/kg) could prevent secondary strokes. Therefore, the present study suggests that ril may reduce the risk of cardiovascular complications in the acute phase after hemorrhagic stroke in the insular cortex without major effects on anxiety/compulsive-like behavior and somatic motor system. As a perspective: next experiments will evaluate the contribution of AT1 and Mas receptors in ril effects.

Key words: stroke, insular cortex, antihypertensive, tachycardia, renin-angiotensin system

761006 ANGIOTENSIN-(1-5) CONTRACTS MOUSE TORACIC AORTA: EVIDENCE FOR A NEGATIVE MODULATORY ROLE OF ENDOTHELIUM-DERIVED NO AND MRGD RECEPTORS

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Angiotensin -(1-7) is considered a biologically active peptide of the renin-angiotensin system. Several studies have expanded the knowledge about the RAS by adding new metabolites of Ang -(1-7), including the pentapeptide angiotensin-(1-5). However, previous studies have provided conflicting evidence regarding the relevance of Ang-(1-5) in vascular function. The vascular response to Ang-(1-5) was evaluated in mouse aortic rings (C57Bl/6J and MrgD -/-). Unexpectedly, Ang 1-5 induced aortic contraction. This effect was increased in endothelium denuded vessels and not changed when the MAS receptor antagonist A779 was added to the organ bath (CT: 3.269±0.159 and A779: 3.262±0.247 mM/ mm). However, in presence of PD 123.319 or the Mas/MrgD antagonists, DPro7-Ang-(1-7) the constritor effect of Ang-(1-5) was enhanced (PD: 4,821±0.339 and DPRO: 5.743±0.220). The Ang-(1-5)-induced contraction was also enhanced in aortic rings taken from MrgD -/- mice in comparion to rings derived from C57BL/6J (CT: 3.269±0.1596 and MrgD: 5.041±0.1514). Addition of PD or D-PRO did not produce a further increase in the contractile effect of Ang-(1-5) in rings of MrgD KO mice. Inhibition of Nitric oxide synthase by L-NAME potentiated the vasoconstrictor effect of Ang -(1-5)(CT: 3.269±0.1596 and L-NAME: 5.321±0.2130). This potentiation was importantly attenuated in aortic rings of MrgD KO mice (MrgD: 5.041±0.1514 and L-NAME: 6.046±0.2258). Our results suggest an important modulatory role for MrgD receptors in the effect of Ang -(1-5) in mouse aorta, possibly involving endothelium-derived NO. Angiotensin -(1-7) is considered a biologically active peptide of the renin-angiotensin system. Several studies have expanded the knowledge about the RAS by adding new metabolites of Ang -(1-7), including the pentapeptide angiotensin-(1-5). However, previous studies have provided conflicting evidence regarding the relevance of Ang-(1-5) in vascular function. The vascular response to Ang-(1-5) was evaluated in mouse aortic rings (C57Bl/6J and MrgD -/-). Unexpectedly, Ang 1-5 induced aortic contraction. This effect was increased in endothelium denuded vessels and not changed when the MAS receptor antagonist A779 was added to the organ bath (CT: 3.269±0.159 and A779: 3.262±0.247 mM/ mm). However, in presence of PD 123.319 or the Mas/MrgD antagonists, DPro7-Ang-(1-7) the constritor effect of Ang-(1-5) was enhanced (PD: 4,821±0.339 and DPRO: 5.743±0.220). The Ang-(1-5)-induced contraction was also enhanced in aortic rings taken from MrgD -/- mice in comparion to rings derived from C57BL/6J (CT: 3.269±0.1596 and MrgD: 5.041±0.1514). Addition of PD or D-PRO did not produce a further increase in the contractile effect of Ang-(1-5) in rings of MrgD KO mice. Inhibition of Nitric oxide synthase by L-NAME potentiated the vasoconstrictor effect of Ang -(1-5)(CT: 3.269±0.1596 and L-NAME: 5.321±0.2130). This potentiation was importantly attenuated in aortic rings of MrgD KO mice (MrgD: 5.041±0.1514 and L-NAME: 6.046±0.2258). Our results suggest an important modulatory role for MrgD receptors in the effect of Ang -(1-5) in mouse aorta, possibly involving endothelium-derived NO.

Key words: angiotensin-(1-5), L-NAME, vasoconstriction.

EXERCISES AND SPORTS

743066 **THERAPEUTIC EFFICACY OF HPß-CD-ANGIOTENSIN-(1-7) ORAL FORMULATION IN MUSCLE INJURY RECOVERY IN RAT**

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Objective: to evaluate the therapeutic effect of oral treatment with HPß-CD-angiotensin-(1-7) (Ang-(1-7)) formulation on muscular recovery following laceration injury. Design: Wistar rats were divided into four experimental groups: Control (n = 10); HPß-CD-angiotensin-(1-7) (Ang-(1-7)) (n = 10); Muscular injury + HPß-CD (inclusion complex only) (MI+Placebo) (n = 24) and Muscular injury + HPß-CD-angiotensin-(1-7) ((MI+Ang-(1-7)) (n = 24). After 7 or 21 days of treatment, physical performance, histological parameters, and pro- and anti-fibrotic gene expression were assessed. Results: The MI+Ang-(1-7) group exhibited enhanced control of the inflammatory phase and reduced deposition of collagen type I and III compared to MI+Placebo. Gene expression of connective tissue growth factor (CTGF) revealed that MI+Ang-(1-7) animals demonstrated decreased pro-fibrogenic factors, along with an increased expression of proteins associated with the blockade of pro-fibrotic pathways. In the treadmill exercise test, MI+Ang-(1-7) animals also demonstrated superior physical performance across all observed post-treatment time points. Conclusion: the oral treatment with Ang-(1-7) is effective in the recovery and control of muscular injuries, with an emphasis on fibrotic lesions, while preserving muscular tissue functionality and physical performance.

Key words: Muscle injury, inflammation, fibrosis, angiotensin, connective tissue growth factor.

743113 **ORAL HPß-CD-ANGIOTENSIN-(1-7) ADMINISTRATION EFFECTS IN MUSCLE RECOVERY OF RECREATIONAL CYCLISTS.**

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Introduction: The positive effects of Angiotensin-(1-7) have been demonstrated in protecting against acute and chronic skeletal muscle injuries in various experimental models. These benefits extend to conditions like muscle weakness associated with aging, immobilization, or dystrophy. Aim: We explore the impact of an oral formulation, HPß-CD-Angiotensin-(1-7), on muscle recovery following damage induced by eccentric exercise. Methods: Five recreational athletes (age: 33.4 ± 9.8 years, body mass: 75.86 ± 8.85 kg and height: 177.2 ± 8.07 cm), involved in training programs for at least one year, participated in this crossover design study. Subjects received either HPß-CD-Ang-(1–7) (2 mg) or HPßCD-Placebo in capsules, administered 48 hours (one capsule each 24 hours) after a muscle-damaging protocol, which consisted of squatting (10 series of 10 repetitions at 75% of one maximal repetition). Heart rate (HR) measurements were taken at rest and at peak effort after a physical test (Wingate). Physical performance (PP) was evaluated based on maximal potential during the Wingate test conducted 48 hours after muscle damage. Edema was assessed using ultrasound, and the thickness of the rectus femoris (RF) and vastus lateralis (VL) muscles was recorded in the dominant leg. Statistical analysis: Data were analyzed using Jamovi Software, version 1.1 (The Jamovi Project, 2.3.26/2023). Results: Preliminary results indicate no significant differences in PP between the two conditions: 211±22 watts (HPß-CD) vs. 192±49 watts [(HPß-CD-Ang-(1-7)]. HR did not differ between the two conditions, both at rest and during peak effort. Edema thickness measurements showed 48 hour after injure was not different for RF [1.92±0.5 cm (placebo) vs. 1.89 ± 0.1 cm (Ang-(1-7))] and for VL [2.29±0.3 cm (placebo) vs. 2.39 ± 0.4 cm (Ang-(1-7))].Conclusion: These preliminary results from a limited sample size (n=5 participants) suggest that oral treatment with HPß-CD-Angiotensin-(1-7) (administered in two doses) does not have a significant impact on HR, in the physical performance and muscle thickness after muscle damage.

Key words: Muscle injury, angiotensin, eccentric exercise, cyclists.

745685 **THE EFFECT OF ORAL HPß-CD-ANGIOTENSIN-(1-7) ADMINISTRATION ON MUSCLE PAIN IN RECREATIONAL CYCLISTS**

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Introduction: Angiotensin-(1-7) has been shown to have positive effects on skeletal muscles, such as reducing muscle atrophy, antifibrotic effects and apotosis. Aim: To explore the effects of an oral formulation, HPß-CD-Angiotensin-(1-7) on muscle pain after eccentric exercise-induced injury. Methods: Five recreational athletes (age: body mass and height) received HPß-CD-Ang-(1-7) (2 mg) or HPßCD-Placebo capsules, administered 48 hours (one capsule every 24 hours) after a muscle damage protocol, (squatting:10 sets of 10 repetitions at 85% of one repetition maximum). Pain was measure by intensity using a pressure algometer with the pain threshold defined in kilogram-force (kgf) rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) muscles and by analogue pain scale (VAS). The pain measurements were taken at before, 24 and 48 hours after muscle injure. Statistical analysis was carried out in GraphPad Prism software (version 8.0) using the Two-Way ANOVA test to compare the time course of pain. Values of $p < 0.05$ were considered statistically significant. Results: Preliminary results showed a lower pain threshold compared to the before moment in the VL: 4.9±0.1 Kgf (before HPß-CD) vs. 3.5±0.9 Kgf (HPß-CD 48h) and 4.6±0.83 Kgf [(before HPß-CD-Ang-(1-7)] vs. 3.7±0.6 Kgf [(HPß-CD-Ang-(1-7) 48h]. VAS muscle pain showed a difference for RF with HPßCD-Placebo [0±0 cm (before) vs. 1.4±1.1 cm (48h)] and HPß-CD-Ang-(1-7) [0.2±0.44 cm (before) vs. 0.8±1.09 cm (48h)], and also for VL with HPßCD-Placebo [0.8±1.3 cm (before) vs. 2.0±1.0 cm (48h)] and HPß-CD-Ang-(1-7) [0.8±1.3 cm (before) vs. 2.2±0.44 cm (48h)]. Conclusion: Based on the preliminary results of a limited sample size (n=5 participants), they suggest that injure protocol induced pain without differences between HPßCD-Placebo and HPß-CD-Angiotensin-(1-7).

Key words: Angiotensin-(1-7), Muscle Pain, Recreational Cyclists, Oral HPß-CD-Angiotensin-(1-7), algometer

750242 **EFFECT OF SUPPLEMENTATION WITH GAMMA AMINOBUTYRIC ACID ASSOCIATED WITH PHYSICAL EXERCISE ON ANTHROPOMETRIC, BIOCHEMICAL AND HORMONAL PARAMETERS**

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Introduction: The prevalence of obesity has witnessed a significant increase in recent years, contributing to the development of various chronic noncommunicable diseases (NCDs). A recent area of investigation centers on the potential relationship between gamma-aminobutyric acid (GABA) supplementation, heightened growth hormone (GH) secretion, and the reduction of inflammation. Objective: This study aims to evaluate the impact of combining GABA supplementation with physical training on anthropometric, biochemical, hormonal, and physical parameters in obese women. Methodology: The research included 26 female volunteers with obesity who were randomly assigned, using a single-blind approach, to two groups: placebo (n=12) and GABA (n=14). These participants engaged in a three-times-a-week combined training program (involving aerobic and strength exercises) lasting 50 minutes for a duration of 90 days. They ingested a daily pill that was identical in color, size, and smell, containing either 200mg of GABA or a placebo. At two distinct time points (T0 and T90), the volunteers underwent a battery of physical tests and provided blood samples for hormonal and biochemical analyses. Results: Anthropometric data revealed an increase in % body fat in the placebo group after T90, along with a reduction in fat-free mass, while the GABA group maintained their composition. Biochemical parameters showed lower triglyceride levels in the GABA group at T90. There was no significant difference in growth hormone levels between the two groups. Physical tests demonstrated improved hand grip strength after 90 days of training. Notably, inflammatory cytokines did not show any significant effects of the supplementation. Conclusion: These findings suggest that GABA supplementation has a positive effect on maintaining a more favorable body composition without interfering with growth hormone levels, physical performance, or parameters related to inflammation in obese women.

Key words: physical exercise; muscle strength; GABA; obesity.

748925 **ASSESSING THE USE OF CRYPTIC PEPTIDE FROM A SCORPION VENOM PEPTIDE AS AN ANTIOXIDANT AGENT AND PERFORMANCE MODULATOR IN ENDURANCE PHYSICAL ACTIVITY OF ANIMALS**.

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Introduction: Proven benefits of physical activity are the reduction of blood pressure, glycemic control, weight management, treatment of anxiety and depression, among others. Recent studies in hypertensive rats have shown that regular aerobic training can alleviate chronic hypertension by reducing the concentration of AngII and increasing Ang-(1-7). Nevertheless, while light to moderate aerobic exercise offers well-established benefits, endurance physical activity leads to the generation of free radicals and reactive oxygen species, causing an imbalance in the REDOX system. This event is known as the ""overtraining syndrome"", which is detrimental to the health and performance of athletes. The hypothesis of this study is that KPP – a cryptic peptide from the scorpion venom peptide Ts14 – can be used to reduce the associated production of oxidative stress and, eventually, improve performance in endurance physical activity due to its antioxidative and vasodilator effects. Objectives: The aim of this work is to assess the outcomes of KPP oral treatment in an experimental model of endurance physical activity. Methods: Male C57BL/6 mice (n=8) were divided into 2 experimental groups, (a) Control (saline oral treatment) and (b) KPP (KPP 1mg/Kg oral treatment) . A treadmill familiarization test was conducted from Day 1 to Day 5 as follows: Day 1: 3 minutes of rest, 5m/min (5 minutes), 6m/min (3 minutes); Day 2: 3 minutes of rest, 6m/min (5 minutes), 8m/min (3 minutes); Days 3-4: 3 minutes of rest, 5m/min (5 minutes), 8m/min (5 minutes); Day 5: 3 minutes of rest, incremental load test (test ending 10 seconds before the fatigue zone). On Day 8, a single dose of a 60µL solution (1mg/kg of KPP at 10-3 M) or saline was administered 3 hours before the test and followed by an incremental load test (starting at 10m/min and increasing by 3m/min every 3 minutes, ending 5 seconds before the shock zone or 10 seconds before the fatigue zone). The animals were euthanized immediately after the test. Animal experiments were approved by the local Animal Ethical Committee (CEUA-UFMG; protocol 241/2023)Results: Preliminary data indicate that KPP treatment induce marginal exercise improvement, by a slight increase in the exercise time and maximum running speed. Conclusions: More experiments are necessary to test the beneficial aspects of KPP in endurance physical activity. Financial supports: CNPq, Capes and Fapemig.
Key words: Peptide, Scorpion Venom, Antioxidant, Redox, Physical Activity

GUT AND MICROBIOTA

748356 **METABOLIC AND BEHAVIORAL CHARACTERIZATION OF THE CHRONIC TREATMENT OF MICE WITH AN ORAL FORMULATION OF ANGIOTENSIN-(1-7) IN A MODEL OF DYSBIOSIS INDUCED BY ANTIBIOTICS**

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Gut dysbiosis is a condition when occurs an imbalance in the gut microorganisms. This alteration can be caused by feeding, chronical stress, sedentarism, hormonal alterations and other factors. Also, chronical treatment with antibiotics can lead to reduction of gut microbiota, which is associated with various health complications, including metabolic and neuropsychiatric disorders. Recent studies have shown that angiotensin-(1-7) [ang-(1-7)], a bioactive peptide from the renin-angiotensin system, plays a key role in the positive modulation of the intestinal microbiota. This suggests that it may be a potential candidate in the treatment of gut dysbiosis and its comorbidities. The aim of this study is to investigate if the depletion of the gut microbiota by antibiotics can lead to metabolic and behavioral changes in mice and if the treatment with ang-(1-7) can revert these alterations. Male C57BL/6 mice were divided into 4 experimental groups with 5 mice (n=5): NC (Normobiosis + Cyclodextrin [CD]); NA (Normobiosis + Ang-(1-7) included in CD, 60µg/kg); DC (Dysbiosis + CD); DA (Dysbiosis + Ang-(1-7) included in CD, 60µg/kg). Dysbiosis was induced by a single dose of 20 mg/Kg streptomycin (Sigma) per os (P.O.) and provided ampicillin (Sigma) at 1 g/L in drinking water for 2 weeks. Normobiosis animals were treated with saline. One day after the last treatment, mice were subjected to metabolic cage, for the collection of urine, feces, water and feed consumption, or to behavioral tests Marble Burying (MB), Tail Suspension Test (TST), Light Dark Box (LDB) and Elevated Plus Maze (EPM) (CEUA:144/2023). Data were analyzed by two or three-way ANOVA followed by Bonferroni posthoc tests ($p<0.05$ considered significant). Both dysbiosis and treatment with ang-(1-7) did not change the following metabolic parameters: body mass, water and feed consumption, and glycemia. However, gut dysbiosis promoted a decrease in the animals' body adiposity (g) (NC:1.5±0.1; NA:1.3±0.1; DC:1.21±0.1; DA:1.2±0.1). In addition, the treatment with ang-(1-7) increased the urinary osmolarity (mOsm/L) (NC:2282±289.4; NA:2438±192.4; DC:2410±228.1; DA:3913.3±333.2) and the proteinuria (mg/dL) (NC:168.2±16; NA:245±63.9; DC:124.4±22.6; DA:503±136.3), while not promoting any effect on creatinine levels and glycosuria. Additionally, both dysbiosis and treatment with ang-(1-7) did not modify the compulsive-like behavior in MB, depressive-like behavior in TST, and anxiety-like behavior in LDB and EPM. Excepting to body adiposity, dysbiosis and treatment with ang-(1-7) did not significantly modify the metabolic parameters and behavior, but the used ang-(1-7) dose seems to exert effects on renal function. Considering these findings, further research involving a larger number of animals is necessary to gain a better understanding of these results.

Key words: Dysbiosis, Angiotensin-(1-7), metabolism.

749868 **THE EFFECT OF MODULATING THE INTESTINAL MICROBIOTA BY ANGIOTENSIN (1-7) ON METABOLIC AND BEHAVIORAL CHANGES IN C57BL/6 MICE**

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Introduction: Gut dysbiosis is a condition characterized by an imbalance in the gut microbiota, which can be triggered by various factors, including inadequate diet and antibiotic use. This condition can lead to a range of health complications, such as neuropsychiatric disorders and metabolic changes. Therefore, it is crucial to identify new therapeutic targets and treatments that can mitigate gut dysbiosis. In this context, the use of angiotensin-(1-7) [ang-(1-7)], a bioactive peptide that plays a crucial role in regulating blood pressure and has potential therapeutic applications in cardiovascular health, appears promising. Objectives: Investigate whether treatment with ang-(1-7) could influence metabolic and behavioral parameters in mice with dysbiosis induced by a combination of a high-fat diet (HF) and antibiotic therapy. Methods: Male mice at 8 weeks of age were initially divided into two groups: SD, which received a standard diet, and HF, which received a high-fat diet, both maintained for 14 weeks. In the 11th week, animals in the HF group underwent an antibiotic therapy protocol, consisting of the administration of ampicillin (Sigma) at a concentration of 1g/L in drinking water for 2 weeks, along with a single oral dose of 20 mg/animal of streptomycin (Sigma). Concomitantly, the both groups were subdivided into two subgroups: “ang-(1-7)”, which received this heptapeptide included in hydroxypropyl ß-cyclodextrin (CD) via orogastric gavage at a dose of 30 µg/kg ang-(1-7) + 45 µg/kg CD for 21 days, and “CD”, which received HPßCD via orogastric gavage at a dose of 45 µg/kg, also for 21 days. In the 12th week, the mice underwent behavioral tests to assess anxious, compulsive, and depressive behaviors. Subsequently, they were placed in metabolic cages for urine collection. At the end of the experimental period, the animals were euthanized for blood and tissues collection. (CEUA: 144/2023). Results: The consumption of the HF diet for 14 weeks promoted an increase in body weight in the animals from the 7th week, an increase in body adiposity, and blood glucose levels, although it did not alter the animals' food intake. Additionally, the HF diet led to an increase in the weight of the cecum, kidney, and plasma creatinine. Both, the consumption of the HF diet and treatment with ang-(1-7) increased the heart weight of the animals. The hyperglycemic effect of the HF diet was abolished after antibiotic therapy. Neither the combination of the HF diet with antibiotic therapy nor treatment with ang-(1-7) altered the urinary levels of creatinine, urea, glucose, and urinary gamma GT and urinary flow. However, the increased water intake induced by ang-(1-7) was abolished in HF diet animals. There were no behavioral changes observed in any animal group. Conclusions: Preliminary data support the idea that some effects induced by ang-(1-7) are changed due to HF/antibiotics-induced dysbiosis, though more experiments are needed to backup this idea.

Key words: High Fat Diet, Angiotensin-(1-7), Dysbiosis, microbiota

HORMONAL BIOLOGY

750515 **ACTION OF ANGIOTENSIN (1-7) INCLUDED IN CYCLODEXTRIN ORALLY ADMINISTERED IN RATS WITH POLYCYSTIC OVARY INDUCED BY ESTRADIOL VALERATE**

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Introduction: Polycystic Ovarian Syndrome (PCOS) is a multifactorial endocrine condition, whose worldwide prevalence is 6-10%. The main findings are an ovulatory cycles, infertility, menstrual irregularity and hyperandrogenism. The angiotensin 1-7 (Ang (1-7)) is a component of the renin-angiotensin system (RAS). It's the main product of the angiotensin-converting enzyme 2 (ACE2) and the presence in the ovaries of rats have a variable distribution according to the phase of the estrous cycle. In ovaries of rats with polycystic ovary condition (PCO) there are reduced levels of Ang (1-7) and the Mas receptor, in addition to reduced expression of ACE2 mRNA compared to normal ovaries, suggesting inhibition of the ovarian ACE2-Ang-receptor axis. Aim: Evaluate the action of Ang (1-7) included cyclodextrin, orally administered in rats with polycystic ovary induced by estradiol valerate (EV). Methods: Adult female Wistar rats (Rattus novergicus), with regular estrous cycles, at eight weeks of age, were divided into two groups: PCO, treated with intramuscular 2 mg of EV diluted in 0.2 mL of corn oil (vehicle), in a single dose; and control, which received 0.2 mL of vehicle intramuscularly in a single dose. Both groups were divided into two subgroups after 60 days, one treated with 30 µg/kg of Ang (1-7) included in cyclodextrin and the other with 30 µg/kg of cyclodextrin, both diluted in 0.5 ml of filtered tap water, by daily gavage, for 15 days. After treatment, the animals were euthanized by guillotine, after sedation with isoflurane, in estrus. The ovary was weighed and fixed in 4% paraformaldehyde (PFA) solution for 24 hours, and then stored in PBS buffer solution (0.05 mol/L sodium phosphate, pH 7.2-7.4) at 4°C. Then, each sample was placed in an identified cassette, dehydrated, impregnated and embedded in paraffin, cut for mounting slides, stained with Hematoxylin-Eosin and evaluated under an optical microscope for structure quantification. The experimental protocols were approved by the Animal Use Ethics Committee (Comissão de Ética no Uso de Animais - CEUA), protocol number 134/2022. Results: Animals in the PCO group developed ovarian cysts after treatment with VE (0 ± 0 vs. 8.97 ± 1.16. $P<0.0001$. Mean ± SEM), and oral treatment with Ang (1-7) included in cyclodextrin reduced the total number of cysts (11.43 ± 1.82 vs. 6.81 ± 1.32. $P<0.05$. Mean ± SEM). There was a reduction in corpora lutea in animals in the PCO group (12.89 ± 1.25 vs. 5.90 ± 0.96. $P<0.005$. Mean ± SEM), however the effect was not altered by treatment with Ang (1-7) included in cyclodextrin. Healthy follicles did not change between groups. Conclusion: In our study, we found that the administration of Ang (1-7) by gavage reduced the cysts in rats with PCO in an experimental model induced by estradiol valerate. Ang (1-7) has been shown to be a promising drug in the treatment of ovarian cysts resulting from PCOS."

Key words: PCO, Ang (1-7), ovarian histology.

MOLECULAR BIOLOGY

745883 **ASSESSMENT OF GENE EXPRESSION OF MAS AND MRGD RECEPTORS IN THE NASAL EPITHELIUM OF EUTROPHIC AND OBESE INDIVIDUALS INFECTED WITH SARS-COV-2.**

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Introduction: Obesity is a risk factor for a worse prognosis in the evolution of Coronavirus Disease 2019 (COVID-19); however the pathophysiological mechanism of this relationship is not yet well established. The dysregulation of components of the Renin Angiotensin System (RAS) may be a critical factor in this worsening. In SARS-CoV-2 infection, the RAS may be overactivated, hindering the cleavage of angiotensins (Ang) into products with anti-inflammatory effects, culminating in cytokine storm syndrome. In homeostasis, the MAS receptor is activated by Ang (1-7) and the MRGD receptor by alamandine, promoting anti-inflammatory effects. The expression of these receptors may be related to obesity and the morbidities associated with this condition in COVID-19. It is suggested that the imbalance of pro- and anti-inflammatory factors may trigger a more exacerbated pathogenesis in patients with obesity, in relation to eutrophic patients. Therefore, it is essential to recognize the tissue expression of such RAS components and their possible changes in the face of pathological challenges. Aim: To investigate and compare the gene expression of MAS and MRGD receptors in the nasal epithelium of eutrophic and obese individuals infected with SARS-CoV-2. Methods: After approval by the Ethics Committee (CAAE: 55519822.4.0000.5108), participants were divided into groups: case, individuals diagnosed positive for COVID-19 and symptomatic; and control, individuals with a negative diagnosis for COVID-19 and no symptoms. Individuals under 18 years of age, who had received the vaccine 30 days prior and who experienced an infectious condition 15 days prior to collection were excluded. Total RNA was extracted from the nasopharyngeal swab samples, and then complementary DNA was synthesized. Quantification of gene expression levels of MAS and MRGD were analyzed using Reverse Transcription Quantitative Polymerase Chain Reaction (RTqPCR). Data were analyzed by one-way ANOVA or paired t-test, p-value = 0.05 was considered statistically significant. Results: The following were included: (n=18) people with obesity and positive for COVID-19 (OB+); (n=18) eutrophic and positive for COVID-19 (EU+); (n=17) with obesity and negative for COVID-19 (OB-); (n=19) eutrophic and negative for COVID-19 (EU-). There was a decrease in MAS expression comparing: OB- and OB+ ($p = <0.0001$); EU- and EU+ ($p = <0.0001$); OB- and EU+ ($p = <0.0001$). Regarding the MRGD gene, there was a decrease in expression comparing: OB- and OB+ ($p = <0.0001$); EU- and EU+ ($p = <0.0003$); OB- and EU+ ($p = <0.0001$); OB- and EU- ($p = <0.0001$). Conclusions: We demonstrate that both obesity and SARS-CoV-2 infection can reduce the gene expression of MAS and MRGD receptors in the nasal mucosa of adults. The reduction of these components may be associated with a pro-inflammatory environment in people with obesity. More studies will be needed to elucidate mechanisms of this low expression in obese people and those with COVID-19.

Key words: Renin-Angiotensin System; Angiotensin Receptors; Obesity; COVID-19; SARS-CoV-2.

749413 **GENETIC PROFILE OF BRADYKININ-FORMING CASCADE AND VASCULAR ENDOTHELIUM PERMEABILITY REGULATION IN BRAZILIAN PATIENTS WITH HEREDITARY ANGIOEDEMA**

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Introduction: Hereditary angioedema (HAE) is a rare dominant autosomal disease characterized by swelling without urticaria and categorized in three types: quantitative deficiency or dysfunction (types I and II HAE C1-INH) of C1 esterase inhibitor (C1-INH) and HAE with normal C1-INH (HAE nC1-INH). HAE is associated with the dysregulation of bradykinin-forming cascade, causing its overproduction, which promotes increased permeability, leading to AE attacks. The most common form comprehends both HAE C1-INH types and is caused by mutations in SERPING1. HAE nC1-INH is even rarer and related to mutations in F12, ANGPT1, PLG, KNG1, MYOF and HS3ST6, with p.Thr328Lys in F12 mainly identified. However, in several HAE nC1-INH patients, genetic cause is still unknown, and the pathogenesis remains unclear. In addition to clinical symptoms, family history and laboratory tests, the diagnostic of HAE is confirmed by molecular study. Therefore, the investigation of genetic background of HAE is important to understand its pathophysiology and for early diagnosis, essential in the management of HAE, since if not treated correctly, can lead to mortality due to laryngeal edema. Aim: Evaluate the genetic profile of components involved in bradykinin-forming pathway and regulation of vascular endothelium permeability in patients of HAE Brazilian Study Group (GEBRAEH). Methods: A total of 219 subjects (probands and symptomatic or asymptomatic relatives) of 130 unrelated families were classified in HAE types, according to anamnesis and biochemical parameters. Genetic analysis of SERPING1 (78 subjects of 22 families) and exon 9 of F12 (141 subjects of 108 families) was performed by Sanger Sequencing or MLPA technique. In 25 HAE nC1-INH probands which no mutation was found, Whole-Exome Sequencing (WES) was performed, targeting 65 genes possibly related to HAE. Results: In 41 subjects (58.5% females) of 16 HAE C1-INH families, 13 different mutations in SERPING1 were identified in heterozygosity, including mostly missense (38.4%), followed by nonsense (30.7%) variants and two novel variants (a deletion-insertion and a large deletion). In 50 individuals (72% females) of 34 HAE nC1-INH families, p.Thr328Lys was identified in heterozygosity. Furthermore, WES identified 57 rare variants in 35 genes, all of them were found in heterozygosity and mostly are missense (89.6%). Some of them, found in ADGRE2, GRK2, HS3ST6, KNG1, NOS3, RASA1, SERPINA1 and SYTL2 may be candidate variants to be involved in HAE. Conclusions: Our results demonstrate large diversity of mutations in SERPING1 and that around 30% of HAE nC1-INH families carry a mutation which affects FXII function, while in majority of cases, the genetic cause is unknown. Considering the large number of patients undiagnosed, the search for novel variants related to HAE becomes relevant. Elucidating the mechanisms of modulation of AE attacks can increase knowledge for diagnosis and generation of new therapeutic strategies for HAE.

Key words: Genetics, Hereditary Angioedema, Bradykinin, Endothelium.

PHARMACOLOGY AND DRUG DISCOVERY

742610 **ANGIOTENSIN-(1-7) IMPROVES EARLY HOST IMMUNE RESPONSES, AND EXERTS PRO-RESOLVING EFFECTS IN A MODEL OF SEVERE DENGUE INFECTION**

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Introduction: Host immune responses play a significant role in the pathogenesis and severity of dengue. Severe dengue is not only the result of an exacerbated inflammatory response but also a consequence of dysregulated or 'failed' pro-resolving mechanisms. Therapeutic strategies that utilize pro-resolving molecules have great potential for treating acute inflammatory infectious diseases. Angiotensin-(1-7) [Ang-(1-7)], a biologically active peptide of the renin-angiotensin system (RAS), acts on its receptor Mas (MasR) to promote inflammation resolution. Objective: In this study, we sought to investigate the therapeutic potential of Ang-(1-7) during dengue infection. Methods: A129 mice were infected with DENV-2, and specific groups were treated with a solution containing [Ang-(1-7)] at a dose of 100 µg/kg administered subcutaneously (s.c.) twice a day, starting 36 hours post-infection. Euthanasia was performed 3 days after DENV infection (CEUA: 12/2023). Results: DENV infection resulted in increased clinical scores, including body weight loss, thrombocytopenia, and leukocytosis, along with elevated levels of inflammatory mediators in the plasma and spleen, characterizing a cytokine storm. However, therapeutic treatment with Ang-(1-7) effectively prevented the increase in clinical scores and thrombocytopenia induced by the infection. It also positively influenced the numbers of circulating leukocytes in whole blood, primarily by reducing the ratio between granulocytes and lymphocytes. Notably, Ang-(1-7) improved the levels of inflammatory markers in both plasma (CXCL1 and INF?) and spleen (CXCL1, IFN-γ, TGF-ß, IL12, and IL6), while reducing levels of MCPT-1, a cytokine released by mastocytes associated with plasma leakage. Finally, treatment did not have a major impact on viral titers in plasma and target organs (spleen and liver). Conclusion: In summary, our findings suggest that Ang-(1-7) expedites the resolution of inflammation by enhancing lymphocyte numbers and activation, as well as potentiating early pro-inflammatory responses that ultimately lead to a systemic reduction in viral titers and the amelioration of clinical symptoms.

Key words: Dengue; Angiotensin-(1-7); Inflammation; Resolution; Infection

743036 **ACETYL-ANGIOTENSIN (2-7)-AMIDE: AN ACE INHIBITOR WITH POTENT ANTI-HYPERTENSIVE EFFECT**

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Introduction: The angiotensin-converting enzyme (ACE) has two active connection sites located in its catalytic domain. The first active site, known as the N domain, is responsible for the cleavage of angiotensin I into angiotensin II, a potent vasoconstrictor that increases blood pressure. The second active site, the C domain, participates in the manipulation of bradykinin, a vasodilatory peptide. ACE inhibitors are among the most used medications in the treatment of hypertension, but as they inhibit both domains, they are associated with adverse effects. Recently, in vitro studies demonstrated that acetyl-angiotensin (2-7)-amide (Ac-Ang (2-7)-NH2) is a potent ACE inhibitor, resistant to cleavage and with improved selectivity of the ACE C domain. Objective: To investigate whether Ac-Ang (2-7)-NH2 is capable of reducing blood pressure selectively for the C domain. Methods: We used male spontaneously hypertensive rats (SHR) weighing 300g at 3 months of age and Wistar rats control males weighing 400g at 3 months of age. Animals were cannulated for accurate blood pressure (BP) and heart rate (HR), as well as intravenous drug injection. BP and HR were monitored continuously for 1 hour in each animal to establish baseline levels. Subsequently, the animals received intravenous injections of Ac-Ang (2-7)-NH2 in doses of 30 micrograms (SHR N=4; WISTAR N=3) and 6 micrograms (SHR N=5; WISTAR N=3). We recorded an additional six hours of BP and HR determination after the injections. Statistical analysis was performed using a one-way ANOVA (analysis of variance) to compare means between baseline, 30 minutes after peptide injection, and 6 hours after peptide injection, and for comparisons between two groups, one t test was businessman ($p < 0.05$). CEUA: 113/2022. Results: Ac-Ang (2-7)-NH2 significantly lowers blood pressure in SHR after intravenous administration at concentrations of 30µg/kg (BP -35.25 ± 6.22 mmHg vs -1.33 ± 0 .88 mmHg, $p < 0.01$) and 6µg/kg (BP -24.80 ± 6.18 mmHg vs -5.33 ± 3.18 mmHg, $p < 0.01$). However, this peptide had no effect on the blood pressure of normotensive animals, regardless of the concentration administered. Furthermore, no significant changes in heart rate were observed between the SHR group and the normotensive group. Conclusion: These results indicate that Ac-Ang (2-7)-NH2 acts as a potent antihypertensive drug. Its selectivity for the C domain of ACE suggests that it may avoid the adverse effects associated with classical ACE inhibitors, which inhibit both ACE domains and lead to bradykinin accumulation.

Key words: Acetyl-angiotensin (2-7)-amide, Ace inhibitors, Anti-hypertensives, Hypertension, Vasoactive peptides.

751145 **ANGIOTENSIN-(1-7) DECREASES INFECTION-ASSOCIATED LYMPHOPENIA AND IMPROVES MHV-3-INDUCED LUNG DAMAGE**

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Introduction: Exacerbated inflammatory response plays a crucial role in the progression and severity of acute respiratory syndrome (SARS) induced by the murine beta coronavirus MHV-3. The viral infection triggers a robust inflammatory response, leading to acute lung injury and significant impairment of the respiratory function. After lung infection, the virus spread systemically resulting in high viral loads in multiple organs and severe systemic disease, in which cause the death of all mice within 6-7 days post-infection (dpi). In this study, we have evaluated the effect of systemic administration of Angiotensin-(1-7)-[Ang-(1-7)], a peptide of the contra-regulatory arm of the Renin-Angiotensin System (RAS) endowed with anti-inflammatory and pro-resolving properties, on the overwhelming inflammation and tissue damage induced by MHV-3 infection. Methods: We used 6-week-old male C57BL/6 WT mice infected intranasally (i.n.) with 3x103 PFUs of MHV-3. After 12, 24, 36, and 48 hours post-infection (hpi) (short protocol) or by 12/12 h until 96h (long protocol) mice were treated with vehicle (Saline + or DMSO intraperitoneally, i.p.) or Ang-(1-7) (30ug/kg/animal, i.p.). Mice were euthanized three or five days after infection and samples were harvested for analysis of the inflammatory profile in bronchoalveolar lavage. Another group of mice was used to evaluate the lethality rates after Ang-(1-7) treatment of MHV-infected animals. To this, mice were infected with 1×102 PFUs of MHV-3 (i.n.) and treated with vehicle or Ang-(1-7) by 24, 48, 72, 96, and 120 hpi (30ug/kg/animal, i.p.). Results: Treatment with Ang-(1-7) reduced leukocyte infiltration into the alveoli of mice, compared to untreated animals, which was mainly characterized by a decrease in mononuclear cells in both protocols applied. Additionally, Ang-(1-7) treatment reversed the pronounced blood lymphopenia characteristic of MHV-3 infection at the 3dpi and decrease the systemic levels of IL-6 at 5dpi, without effect in virus titters in lungs. Notably, the administration of Ang-(1-7) was able to rescue approximately 50% of mice from MHV-induced lethality and also resulted in a recovery of 20-25% of body weight compared to untreated animals with Ang-(1-7). Conclusion: Our data suggest that treatment with Ang-(1-7) was effective in protecting the host by modulating the inflammatory response, reducing mortality, and promoting weight recovery. Financial support: CAPES, 23038.003950/2020-16; CNPq, PQ-306789/2018/3. and Fapemig, BPD-01010-22/ 30338.

Key words: Angiotensin-(1-7), MHV-3, Lung inflammation

750431 **THE AGIOTENSIN-(1-7)/MASR AXIS MODULATES THE INFLAMMATORY RESPONSE AND BACTERIAL DISSEMINATION IN PNEUMONIA CAUSED BY PSEUDOMONAS AERUGINOSA**

Isabella Zaidan Moreira, Antônio Felipe Silva Carvalho, Laís Cunha Grossi Ferreira, Jéssica Amanda Marques Souza, Edvaldo Souza Lara, Ana Clara Matoso Montuori de Andrade, Camila Cardoso, Fernanda Silva Carneiro, Erick Bryan de Sousa Lima, Adelson Héric Alves Monteiro, Isabella de Lacerda Augusto, Rodrigo Severo Caixeta, Camila Bernardo de Brito, Leonardo Camilo de Oliveira, Celso Queiroz-Junior, Remo de Castro Russo, Maria José Campagnole, Robson Augusto Souza dos Santos, Vivian Vasconcelos Costa, Daniele Souza, Caio Fagundes, Mauro Martins Teixeira, Luciana P. Tavares, Lirlândia Pires de Sousa.

Introduction: Pseudomonas aeruginosa is a common cause of hospital-acquired pneumonia. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and ubiquity. Inflammation triggered by P. aeruginosa infection is necessary for bacterial clearance but must be spatially and temporally regulated to prevent further tissue damage and bacterial dissemination. Inflammation resolution is an active and integrated process that is coordinated by different pro-resolving mediators, including Angiotensin-(1-7) [Ang-(1-7)]. The actions of Ang-(1-7) occur mainly through its binding to the Mas receptor (MasR), a G protein-coupled receptor. Among its anti-inflammatory actions, Ang-(1-7) acting via MasR, reduces the recruitment of leukocytes, and prevents tissue damage related to the overactivation of inflammation. Aim: The present work highlights the importance of the Ang-(1-7)/Mas axis in pneumonia caused by P. aeruginosa. Methods: C57BL/6 wild-type or Mas receptor-deficient mice (Mas-/-) were anesthetized with ketamine/xylazine (80 mg/kg and 10 mg/kg, respectively) and acute lung injury (ALI) was induced by intranasal instillation of 106 CFU of P. aeruginosa (strain PAO1). The bronchoalveolar lavage (BAL), blood and lungs samples were harvested, 24 hours post infection (hpi), to analyze inflammatory parameters, bacterial loads and tissue damage. In a therapeutic protocol, PAO1-infected mice were treated with Ang-(1-7) (30µg/mice, i.p.) or vehicle (saline 0.9%, i.p.). Samples were harvest 48 or 72 hpi, in order to evaluation of the same parameters. Results: Mas receptor-deficient animals developed a more severe form of pneumonia, with increased numbers of neutrophils in the BAL, increased bacterial load, and increased levels of cytokines and chemokines. Overall MasR deficient mice show a more severe pulmonary damage after P. aeruginosa infection displaying higher levels of protein in BAL and fibrin deposition in lungs, although the histopathological score was similar in both genotypes. Interestingly, treatment with Ang-(1-7) was able to decrease neutrophilic infiltration and levels of pro-inflammatory cytokines and chemokines in BAL, lung, and plasma of infected animals. Moreover, the peptide was able to decrease bacterial loads and P. aeruginosa-elicited lung damage, resulting in improvement of lung function. Conclusions: identification of pro-resolving and anti-inflammatory mechanisms of Ang-(1-7) will pave the way for the development of host-directed therapies to promote mechanisms of resistance and resilience to infections.

Key words: Pseudomonas aeruginosa, infection, resolution pharmacology, Angiotensin-(1-7), Mas receptor

748929 **THE USE OF THE PEPTIDE ANGIOTENSIN-(1-7) IN THE TREATMENT OF BREASTFEEDING WOMEN WITH BREAST FISSURES**

Sonuellany Sena de Aguilar, Robson Augusto Souza dos Santos, Daniel Villela.

Introduction: Exclusive breastfeeding until six months of age is recommended worldwide as the best nutritional practice. However, one of the reasons for early weaning is the appearance of breast fissures that cause pain. Ang-(1-7), via the Mas receptor, plays an important protective/repair role during pathophysiological conditions. And precisely, due to its vasodilatory and remodeling effects, the use of Ang-(1-7) peptide can prove to be highly effective in healing breast fissures, as in the successful study by Dr. Kathalee's group Rodgers who evaluated, the effect of the Ang-(1-7) peptide on diabetic foot wounds. Aim: To test the effectiveness of a cream containing the Ang-(1-7) peptide in the treatment of women with breast fissures, in the postpartum period, who were unable to breastfeed. Methods: After approval by the Ethics Committee(CAAE: 51545221.3.0000.5108), a study was carried out with of postpartum women in the immediate postpartum period(n=9), divided into three groups: standard; cream and cream+Ang-(1-7)0.03%. The participants included in the study were aged = 18 years, with residence in Diamantina-MG and sought the milk bank in the postpartum period. To this end, the regression of fissures was analyzed using conventional and temperature photographs, visual analogue pain scale, trauma assessment index and treatment satisfaction scale. For statistical analysis, the non-parametric Manova test of repeated measures with a mixed design and ?² of independence was used. Results: Initially, the correlation between pain and temperature was performed. The results point to statistically significant differences between the measurement moments [F(1)=108.000, p<0.001], with a tendency for patients' pain perception to decrease. Therefore, univariate comparisons and paired comparisons. Finally, differences were identified in the interaction between groups and measurement moments [F(1,534)=3.331,p<0.048]. Paired comparisons confirmed differences between the Cream + Ang-(1-7) group in both the assessment of pain and temperature in relation to the other groups (p<0.001) and no differences between the standard group and the cream group were observed. The results of the exact ?² test indicated that there were no significant differences in the prevalence of trauma types by groups [?²(2)=3.600,p=0.165,VCrammer=0.632]. In relation to the treatment duration, swifter amelioration is observed within the Cream+Ang-(1-7) group. Of the Standard group, 66.5% improved after 7 days and 33.5% after 9.5 days; in the Cream group,100% improved 7 days after the first assessment and in the Cream+Ang-(1-7) group, 33.5% improved after 3.5 days and 66.5% after 7 days. Conclusions: This study demonstrated that the compound combined with 0.03% of Ang-(1-7) peptide exhibited superior efficacy compared to the control groups. Consequently, it underscores the need for more extensive studies using this peptide base. These findings also offer potential alternatives currently used compounds.

Key words: Angiotensin-(1-7); Peptide; Breast Fissures

749012 **CHRONIC APOCYNIN TREATMENT IMPROVED CAVEOLAE FUNCTION IN SHR AORTIC ENDOTHELIAL CELLS**.

Mariana Santana Quirino da Silva, Simone Regina Potje, Cristina Antoniali Silva.

Endothelial cells from Spontaneously Hypertensive rats (SHR) have lower number of caveolae. It was already shown that endothelial function is impaired in blood vessels of SHR. Treatment with methyl-ß-cyclodextrin, a caveolae disruptor, impaired endothelial function in rat blood vessels. Apocynin is an antioxidant drug with antihypertensive effect. Chronic treatment of SHR with Apocynin prevented hypertension and endothelial dysfunction. Moreover, Apocynin treatment induced increased expression of nitric oxide synthase (eNOS) and ATII receptor in the SHR aorta. The objective of this study was to evaluate the effect of chronic treatment with Apocynin on the function of caveolae in the aorta of SHR. SHR and Wistar were used in this study (FOA-CEUA 0097-2021). Animals were treated with Apocynin from the 4th to the 10th week of life (30mg/Kg, diluted in drinking water). Aortic rings were isolated from Wistar and SHR rats, treated or not with apocynin. Concentration-response curves to ACh were evaluated in presence or absence of dextrin (10mmol/L for 60 minutes). NOx (NO2- and NO3-) concentration in aortic tissue was measured in the presence and absence of dextrin. The eNOS and Caveolin-1 (cav-1) expression were evaluated in HUVEC treated with Apocynin (100 µmol, 60 minutes). Treatment with Apocynin reduced blood pressure in SHR and had no effect on Wistar rats. Endothelial dysfunction was prevented in the SHR treated with Apocynin. Dextrin impaired vasodilation responses to ACh in the aortas of both groups, however in aortas from SHR treated with Apocynin, this effect of dextrin was not observed. The NOx concentration was reduced in SHR aorta when compared with Wistar rat aortas. Dextrin reduced NOx concentration in aortas of Wistar rats but had a non-significant effect on SHR aortas. Aortas from SHR treated with Apocynin showed an increase in NOx concentration and dextrin had no effect on it. HUVEC treated with Apocynin showed higher expression of eNOS and Cav-1. These data suggested that Apocynin might be preventing the dextrin damage to caveolae in endothelial cells of SHR. Furthermore, Apocynin was able to modulate the caveolae signaling functions in endothelial cells, improving endothelial function in SHR aorta. In the next steps, we will investigate the mechanisms by which Apocynin improves the structure, integrity and signaling functions of caveolae in SHR endothelial cells. Apoio Financeiro: FAPESP (Processo 2011/20998-0, 2017/18436-0), CAPES (código 001)

Key words: Caveolae, Apocynin, SHR, Aorta, Dextrin

749380 **STUDY OF THE MECHANISMS INVOLVED IN THE DECREASED ENDOTHELIN-1 SIGNALING IN RESISTANCE ARTERIES OF SPONTANEOUSLY DIABETIC MICE**

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Introduction: Diabetes Mellitus (DM) is one of the main risk factors for macro and microvascular complications, such as peripheral arterial disease, retinopathy, ischemia and limb amputation, which are largely responsible for reducing the quality of life and life expectancy of individuals with metabolic disorders. The hyperglycemic state generates endothelial dysfunction and decreases the endothelium's ability to respond to humoral and mechanical stimuli. The changes produced by hyperglycemia in the release of endothelium-derived dilator and constrictor factors compromise the fine adjustment of vascular tone, with a consequent increase in peripheral vascular resistance, contributing to damage to organs such as the kidneys, heart, eyes and brain. Diabetic patients and experimental models of streptozotocin-induced DM present increased plasma concentrations of endothelin-1 (ET-1), as well as decreased vascular contractile response to ET-1. Aim: To investigate whether increased plasma concentration of ET-1 reduces the contractile response mediated by type A endothelin receptors (ETAR) by increasing its internalization and degradation. Methods: We used mesenteric resistance arteries from spontaneously diabetic mice (db/db) and respective control mice (db/+). Arteries were exposed to ET-1, and the contractile response and desensitization of the ETAR were tested using vascular reactivity assays (2nd branch of the mesenteric arteries), in the presence of vehicle (V) or paroxetine (PX, inhibitor of the phosphorylation and internalization of ETAR. Results: ET-1 potency [(pEC50) db/+: 9 ± 0.1; db/db: 8.3 ± 0.09; ($p < 0.05$; n = 7-9)], as well as the maximum contraction [(EMax) db/+: 168% ± 6.8; db/db: 136.2 % ±12.8 ($p < 0.05$; n = 7)] were lower in the db/db group when compared to the control group. The decay constant of the ET-1 response was greater for db/db animals when compared to control animals [db/+: 1.39 x 10^{-2} ± 0.2; db/db: 3.2x10^{-2} ± 0.7; ($p < 0.05$; n=9)]. Arteries of the db/db group incubated with paroxetine exhibited recovery of ET-1 potency [(pEC50) db/db PX: 10.1 ± 0.6; (n=2)] and decay time (db/db PX: 0,36±0,3 x10^{-2}; n = 3). Conclusion: Paroxetine recovered ET-1 vascular responses (maximum effect and potency) of db/db mice. Furthermore, the inhibition of ETAR phosphorylation and internalization delayed the desensitization of the contractile response to ET-1, which was faster in db/db. Therefore, increased ETAR internalization, to be confirmed by molecular experiments, is involved in decreased ET-1 signaling in mesenteric arteries of spontaneously diabetic mice. Financial Support: FAPESP (2023/09528-0), CAPES and CNPq. Approved by the Ethics Committee on Animal Use (CEUA FMRP-USP 1119/2022).

Key words: diabetes; desensitization; endothelin-1; vascular system; ETAR

749610 **CHARACTERISATION OF THE MOLECULAR INTERACTIONS BETWEEN ANGIOTENSIN-(1-5) AND THE ANGIOTENSIN AT2-RECEPTOR**

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Introduction: Recently we characterized Ang-(1-5) as a new biologically active component of the protective arm of the RAS and as a potent endogenous AT2R agonist. However, the molecular mechanisms of the AT2R/Ang-(1-5) interaction remains unknown. Aim: To elucidate the molecular mechanisms of the interaction between Ang-(1-5) and the AT2R. Methods: Molecular docking simulations were performed based on the crystal structure of Ang II bound to the AT2R (PDB: 6JOD). The top two docked conformations were used to conduct a 1μs molecular dynamics simulation. To determine those amino acids of Ang-(1-5) which are critically involved in the Ang-(1-5)/AT2R interaction, an Ala-Scan of Ang-(1-5) was performed. To test the importance of certain residues of the AT2R for the interaction with Ang-(1-5) as determined by in silico simulations (Arg185, Asp297, Met128 and Lys215), these residues were mutated to Ala by site-directed mutagenesis. The impact of these mutations on the efficacy of stimulation of the AT2R by Ang-(1-5) was determined by DAF-FM fluorescence-based, semi-automated nitric oxide (NO) measurements in CHO cells transfected with the wildtype or the mutated AT2Rs. Results: Molecular docking simulations revealed that Ang-(1-5) binds to the AT2R in two different poses, one in a similar orientation as previously resolved for Ang II (PDB: 6JOD) (Conf1) and a second one located deeper in the binding pocket (Conf2). Molecular docking and dynamics data suggested that both Conf1 and Conf2 are similarly involved in AT2R activation by Ang-(1-5), although Conf1 displayed a modestly higher stability when compared to Conf2. Ala-Scan of Ang-(1-5) demonstrated that residues 1, 2 and 5 – corresponding to Asp, Arg and Ile – are important for Ang-(1-5)-mediated AT2R activation, as the corresponding Ala-analogues elicited a significantly weaker NO release from AT2R-CHO when compared to native Ang-(1-5). Site-directed mutagenesis of AT2R Arg185 (important residue in Conf2) and Asp297 (important residue in Conf1) revealed that both residues are essential for the Ang-(1-5)/AT2R interaction, as the response to Ang-(1-5) in AT2R-Arg185Ala-CHO and AT2R-Asp297Ala-CHO was significantly weaker when compared to AT2R-CHO. Lys215 does not seem to be involved in an interaction with Ang-(1-5), as Ang-(1-5)-mediated NO release was comparable in AT2R-Lys215Ala-CHO and AT2R-CHO. Interestingly, Met128 somehow hinders activation of the AT2R by Ang-(1-5), as a significantly stronger NO release by Ang-(1-5) was observed in AT2R-Met128Ala-CHO. Conclusion: In silico and in vitro data provided in this study support that Ang-(1-5) is an AT2R agonist. Our data further provide evidence that the binding mode of Ang-(1-5) to the AT2R is similar to Ang II (Conf1), but that in addition the peptide also interacts with the AT2R in a distinct way from Ang II binding (Conf2). Ang-(1-5)/AT2R interactions can potentially be exploited as a template for the design of a novel class of AT2R agonists.

Key words: AT2-receptors, Ang-(1-5), RAS

749815 **EFFECTS OF THE ADMINISTRATION OF CYCLIC ANGIOTENSIN-1-7 IN PREGNANT WISTAR RAT SUBJECTED TO A HYPERLIPIDIC DIET ON BIOCHEMICAL PARAMETERS IN ADULT OFFSPRING**

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Introduction: Malnutrition on the part of the mother can trigger changes that will have repercussions throughout life, generating impacts on the metabolism of the offspring. Ang-(1-7), a peptide from the Renin – angiotensin system, counterbalances the effects mediated by the classic conversion of angiotensin into angiotensin II (deleterious effects resulting from the stimulation of AT1 receptors) by the angiotensin-converting enzyme (ACE). Ang-(1-7) appears as a regulator of the renin-angiotensin system. In order to delay its degradation, Ang-(1-7) is stabilized by lanthionine through thioether bridges introduced into the peptide. Objectives: Our question is whether, when administered throughout the gestational period, in rats on a high-fat diet, cAng-(1-7) can improve biochemical aspects in adult offspring. Methodology: The work was developed under protocol 5722071222. In the P generation, 48 female Wistar rats (Rattus novergicus), 21 days old, were randomly assigned to receive a high-fat diet (standard Nuvilab® diet plus 30% lard Aurora®) or standard Nuvilab® diet, from weaning and saline or cAng(1-7). All animals received diet and drinking water ad libitum. Upon reaching the age of 90 days, females were placed to mate with males fed a standard Nuvilab® diet. cAng-(1-7) or saline was administered daily, during the gestational period after confirmation of pregnancy through vaginal smear, subcutaneously and at a dose of 6 ug/kg. The F1 generation animals were males, fed a standard Nuvilab® diet, divided into 4 groups: F1 control: offspring of females fed the standard Nuvilab® diet and receiving saline; Control-Angiotensin 1-7: offspring of females submitted to the standard Nuvilab® diet and which received cAng-(1-7); Hyperlipidic F1: offspring of females submitted to a high-fat diet and receiving saline; Hyperlipid-Angiotensin 1-7: offspring of females submitted to a high-fat diet and receiving cAng-(1-7). Serum assessments: At 90 days of age, the F1 generation was euthanized by decapitation and 5 ml of blood was removed for biochemical analyzes following the manufacturer's recommendation (LABTEST®). Statistical analysis: The data were subjected to the normality test, and analysis of variance (ANOVA) followed by the Newman-Keuls test, with statistical significance at $p<0.05$: Results: We saw a decrease in glucose concentrations ($F(1, 44)= 37.09$, $p<0.001$), triglycerides ($F(1,44)= 9.10$, $p<0.005$), cholesterol ($F(1,44)= 43.41$, $p<0.001$), direct bilirubin ($F(1,44)= 52.06$, $p<0.0001$) and creatinine ($F(1,44)= 9.06$, $p< 0.005$) in the offspring of mothers treated with cAng-(1-7) . We saw increased concentrations of urea ($F(1,44)=37.17p<0.0001$) in the offspring of mothers subjected to administration of cAng-(1-7), indicating a possible adverse effect of the peptide. Conclusion: Our work indicates that cAng-(1-7) has properties to minimize the harmful effects caused by a high-fat maternal diet, attenuating metabolic disorders in the offspring of these animals.

Key words: Renin-Angiotensin System; metabolic syndrome; fetal programming

750216 **DISCOVERY MECHANISM OF ACTION OF LOSARTAN AS AN ACE INHIBITOR THROUGH QUANTUM BIOCHEMISTRY**

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Losartan (LST) is a potent and selective Ang II type 1 receptor antagonist widely used in the treatment of hypertension. The formation of Ang II is catalyzed by the angiotensin I-converting enzyme (ACE) through proteolytic cleavage of Ang I, which is involved in the control of blood pressure. In our previous work, we demonstrated the competitive binding assay between lisinopril (LPR) and LST to the CHO-ACE with respective IC50, 0.80± 0.02 and 0.40± 0.17. An understanding of the interaction of LST and ACE is important to assist in the development of new drugs for hypertension therapy. Despite the vast literature on the relationship of LST with the renin–angiotensin system, the actions of LST on the sACE enzyme are so far poorly understood. Methods In view of this, we investigated how losartan can interact with the sACE enzyme to block its activity and intracellular signaling. After performing docking assays following quantum biochemistry calculations using losartan and sACE crystallographic data, we report that their interaction results reveal a new mechanism of action with important implications for understanding its effects on hypertension. We used the N domain of the human sACE complexed with the inhibitor LPR (PDB ID 2C6N). To identify potential binding sites, molecular docking was carried out employing automated docking in the AutoDocking Vina program. The ligand used was optimized LST, and the target was sACE after the removal of the LPR in the LPR–sACE complex. The structures of the LPR–sACE complex and LST–sACE complex were used as inputs for calculations of interaction energies of sACE ligands (LPR and LST) with all amino acids of the sACE. The interaction energies were estimated using the MFCC (Molecular Fractionation with Conjugate Caps) method considering the caps as the neighboring amino acids with the broken peptide bond completed with a hydrogen atom. Results: The sum of the interaction energies of all the amino acids that are at a maximum distance of 3.0 A from the ligands is responsible for the interaction energy of approximately -150 kcal.mol-1 of both LST and LPR, caused mainly by the interaction with the amino acids Gln259, Lys489, Thr358, Glu262 and His331 with LST and His491, Ser333,Thr358,His331,Tyr501 and Phe490 with LPR. The interaction energies of sACE amino acids and ligands (LST and LPR) vary between -55 kcal.mol-1 and 10 kcal.mol-1. The BIRD analysis demonstrated that the molecular structures of LPR and LST activate different residues within the binding pocket. We highlight the residue Lys489, which has strong interaction energy with both LPR and LST, with values exceeding 50 kcal.mol-1 for LPR and 30 kcal.mol-1 for LST. The absolute value of the total sACE interaction energy suggests that LST is a potent sACE inhibitor molecule. Conclusion: The quantum biochemistry techniques used in this work contribute to explaining and unraveling new mechanisms of action of losartan that are already widely used in medicine today.

Key words: Losartan, ACE inhibitor.

748528 **SYNTHESIS AND BIOPHYSICAL STUDIES OF AMIDATED ANGIOTENSIN I AND II: MEMBRANE PEPTIDE INTERACTION AND CARDIOVASCULAR CHANGES BY SYSTEMIC INFUSION**

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Introduction: The Renin-Angiotensin-Aldosterone (RAAS) system generates the reactions, which provide balance of blood pressure, amount of sodium and water in the body. The secreted renin cleaves the N-terminal portion of angiotensinogen resulting in the angiotensin I (DRVYIHPFHL), which by the action of angiotensin converting enzyme (ACE) is converted to angiotensin II (DRVYIHPF). Objectives: The present work aimed to investigate the influence of the amidation of angiotensin I and II in the membrane interaction. Materials and methods: The wide-type and amidated peptides were synthesized by the solid-phase peptide synthesis method via Fmoc strategy, purified by high performance liquid chromatography (HPLC) and characterized by mass spectrometry (Electrospray - ESI). Affinity and interaction were evaluated in the presence of phospholipid membrane models of POPC and POPG by electrochemical impedance spectroscopy, inserting the spacer containing three alanines and one cysteine, in the N- and C-terminal regions. Finally, tests of vascular reactivity and cardiovascular changes were performed by systemic infusion in Wistar rats. Results and conclusions: The results indicated that the CAAA–Angio I amide sequence promotes higher values of charge transfer resistance when compared to the Angio I–AAAC amide, probably due to the lower impediment found by the cysteine binding to the gold electrode. Resistance had its most pronounced effect on zwitterionic POPC then anionic POPC: POPG LUVs, in agreement with the literature, since angiotensin act in eukaryotic cells. In addition, the CAAA–Angio I carboxy showed virtually null resistance to charge transfer in the presence of the POPC vesicles while CAAA–Angio I amide showed increased resistance to charge transfer in similar conditions. Therefore, the decrease of evaluated the vascular activity of Angio I and II amide could be related to its higher affinity to the zwitterionic membrane.

Key words: Bioactive peptides, Amidation, Peptide-membrane interaction.

RENAL PHYSIOLOGY

703627 **REDUCED RENAL HEPCIDIN CLEARANCE CONTRIBUTES TO ANEMIA IN ANGIOTENSINOGEN DEFICIENT MICE**

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Background: The lack of Angiotensin II due to genetic deletion of the renin-angiotensin system (RAS) induces anemia in mice. Aim: The major goal was to further characterize the mechanism of anemia establishment. Methods: Adult male FVB/N mice lacking the RAS precursor protein angiotensinogen (Agt-KO) were used for hematological analyses, plasma and tissue iron measurements, plasma iron balance markers, plasma hepcidin quantification and mRNA quantification of relevant iron balance genes in target organs. Results: Hematological analyses of Agt-KO mice revealed anemia, as expected. Interestingly, Agt-KO erythrocytes were microcytic indicating a possible iron deficiency. Further experiments with plasma of Agt-KO confirmed iron deficiency by reduced levels of circulating iron. In addition, plasma ferritin levels were reduced, and liver and spleen levels of iron were depleted in the anemic line, confirming a systemic iron deficiency. To know if the RAS influences iron homeostasis, we quantified the hepatic mRNA levels of hepcidin which is the major regulator of duodenal iron uptake. Hepcidin gene expression was down regulated exactly as expected in conditions of iron deficiency. However, plasma hepcidin levels were quantified by a specific ELISA and revealed that hepcidin accumulates in blood. Finally, markers of glomerular filtration (urea and creatinine) were measured in plasma of Agt-KO. Similar to previous studies these markers were elevated in Agt-KO plasma. Overall, the data shows that hepatic hepcidin production in Agt-KO is downregulated but its main route of excretion is impaired causing plasma accumulation and consequently iron deficiency. Conclusion: Iron deficiency based on impaired hepcidin clearance contributes to the anemic phenotype observed in rodent models lacking Ang II with impaired glomerular filtration rate, like Agt-KO.

Key words: iron deficiency, erythropoiesis, angiotensin, glomerular filtration

747400 **ACTIVATION OF ANG II/AT1R AXIS MEDIATES THE TUBULAR INJURY ASSOCIATED WITH PROTEINURIA OBSERVED IN THE EARLY STAGE OF DIABETIC KIDNEY DISEASE**

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Introduction: Diabetic kidney disease (DKD) is a progressive kidney disease that involves kidney dysfunction associated with the development of proteinuria, which is the result of glomerular and/or tubular injuries. Some studies indicate that in the early stage of DKD, there is development of tubular proteinuria without previous glomerular changes. However, the mechanisms involved in this process need to be elucidated. In this context, it has been shown that inhibitors of the renin-angiotensin system (RAS) have the potential reno-protective effects in kidney diseases. Aim: The main goal is to investigate the potential involvement of RAS components in the development of tubular injury and proteinuria observed in the DKD pathogenesis. Methods: Were used rats Wistar male (8-10 weeks old) for generating the DKD model induced by streptozotocin (STZ, 55mg/kg). When indicated, animals were treated with oral disease of losartan [angiotensin II type 1 receptor antagonist (AT1R)]. Four experimental groups were generated: 1) CTL, normoglycemic rats (n=5); 2) STZ, diabetic rats (n=4); 3) STZ+LOS, diabetic rats treated with losartan (n=5). After 8 weeks of injection with STZ, the animals were placed in metabolic cages, for the analysis of different clinical and renal function parameters (CEUA-UFMG #100/2023). To analyze renal functional parameters, biochemical analysis of plasma and urine was performed using colorimetric kits. Furthermore, histological analysis of kidney tissue was performed. Results: We observed an increase in blood glucose by 200% and urinary flow by 10 times in the STZ group compared to the CTL group, and simultaneous treatment with losartan does not alter this effect (STZ+LOS group). Analyzing the glomerular function parameters (plasma creatinine and creatinine clearance), no significant changes were observed between the experimental groups. The fractional glucose excretion (FEglucose) was increased 20-fold in the STZ group when compared to CTL group. The treatment with losartan potentiated the diabetes-induced increase in FEglucose (30-fold). Furthermore, we observed an increase in the level of proteinuria (mg/24h, a known marker of renal injury) and urinary Gamma-glutamyl transferase (Gamma-GT, a specific marker of tubular epithelium injury) activity in the STZ group compared to the CTL group. Interestingly, the treatment with losartan significantly attenuated these deleterious effects induced by diabetes (STZ+LOS group). No significant changes were observed in the glomerular structure in any experimental group. However, we observed significant tubular injury in the STZ group which has been ameliorated by losartan treatment (STZ+LOS). Conclusion: Taken together, our preliminary findings indicate that, in the early phase of DKD, diabetes induces the activation of a component of the RAS, specifically the Ang II/AT1R axis, promoting tubular injury and dysfunction associated with the development of proteinuria. Financial support: CNPq, FAPEMIG" Diabetic kidney disease, kidney disease, hyperglycemia, tubular damage, proteinuria, renin-angiotensin system, angiotensin II, losartan, AT1R.

747401 **CHRONIC EXPOSURE TO HYPER-PALATABLE DIET PROMOTES A SUBCLINICAL KIDNEY DISEASE ASSOCIATED WITH TUBULAR PROTEINURIA AND PROXIMAL TUBULE EPITHELIAL CELL INJURY IN A MURINE MODEL.**

Mariana Coelho Moraes, Maria Aparecida Ribeiro Vieira, Diogo de Barros Peruchetti, Luciene Bruno Vieira.

Obesity has become a prevalent condition around the world over the past few decades. There are many causes for obesity development, but one important factor is the increased consumption and wide availability of highly palatable foods, rich in sugar and fat, such as the cafeteria diet, a hyperpalatable diet. Furthermore, high-fat diets (HFD) are accompanied by increased oxidative stress in liver, kidney, and heart tissues, as well as functional and metabolic changes. The pathological features of obesity-induced kidney disease are complex, and the main mechanisms are poorly understood. Although the deleterious consequences of HFD have been documented, changes in kidney morphology and function after chronic use are still unclear. However, mechanisms underlying this process need to be investigated. Aim: To evaluate the potential deleterious effects of chronic exposure to a cafeteria diet (high sugar and butter-HSB diet) on proximal tubule epithelial cells (PTECs) and the possible correlation with the development of tubular proteinuria in a mice model. Methods: Ten-week-old male C57BL/6J mice were randomly divided into two experimental groups: 1) mice fed with a HSB diet (HSB: Carbohydrates: 36%; Proteins: 16%; Lipids: 48%; 4.9Kcal/g) (n=6); or 2) mice fed with control diet (AIN 93G: Carbohydrates: 64%; Proteins: 20%; Lipids: 16%; 3.9 Kcal/g) (n=6). After 11 weeks feeding period, mice were allocated in metabolic cages for 24h-urine collection, and then they were euthanized to obtain serum and kidney samples. The characterization of the obesity model, kidney morphological features, as well as plasma and urine analyses were performed (protocol CEUA-UFMG 71/2023). Statistical analysis was performed using the Prisma 8.0 statistical program (GraphPad, CA, USA). The difference between the two experimental groups was measured by the unpaired t-test. Statistical significance was obtained as $P<0.05$. Results: A significant increase in body weight was observed ($t12=7.296$, $p<0.0001$), adiposity index ($t8 =7.208$, $p<0.0001$), and blood glucose levels ($t9=2.400$, $p<0.03$) in HSB group as compared to AIN93G group, indicating a successful induction of obesity in our model. Regarding renal function, no changes were observed in serum creatinine and blood urea nitrogen (BUN) levels, markers of glomerular function, in any experimental group. Interestingly, the HSB group presented significant proteinuria ($t10=2.728$, $p<0.02$) and high levels of ?-glutamyl transferase ($t7=2.470$, $p<0.04$) in the urine as compared to the control group, indicating PTECs injury and tubular proteinuria. Conclusion: Our data suggest that 12 weeks feeding period with an HSB diet promotes a subclinical state of kidney disease as induces a development of tubular proteinuria associated with PTECs injury without changing glomerular function. These results are interesting in the context of the mechanisms that may underly obesity-induced kidney diseases.

Key words: Subclinical kidney disease, Hyperpalatable foods.

748505 **HIGH SYSTEMIC LEVELS OF ANGIOTENSIN-(1-7) MODULATE RENAL DYSFUNCTION OBSERVED IN THE EARLY PHASE OF DIABETIC KIDNEY DISEASE.**

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Introduction: Diabetic Kidney Disease (DKD) is characterized by the progressive renal dysfunction associated with hyperglycemia. The deleterious effects of hyperglycemia on kidney cells are a critical factor for DKD development. In this context, the hyperactivation of the Renin-Angiotensin System (RAS) has been associated with kidney dysfunction in DKD. However, the potential effect of angiotensin 1-7 (Ang-(1-7)), from the alternative arm of RAS, within DKD, still needs to be elucidated. Aim: We aimed to verify the possible role of systemic Ang-(1-7) in the development of DKD. Methods: We used 16-week-old male TGR (L-3292), which has a higher systemic level of Ang-(1-7), and Sprague Dawley rats as the control group. Type 1 diabetes mellitus was induced via a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg). Four experimental groups were generated: 1) SD-ND, normoglycemic Sprague Dawley rat (control, n=4); 2) SD-D, diabetic Sprague Dawley rat (n=3); 3) L3292-ND, normoglycemic transgenic rat (n=5); 4) L3292-D, diabetic transgenic rat (n=5). In 3rd week after the STZ injection, the animals were housed in metabolic cages to perform the renal function analysis (CEUA-UFMG#100/2023). Results: Diabetic groups had higher water intake and urinary volume when compared with normoglycemic groups. Moreover, these changes were potentiated in the L3292-D group than in the SD-D group. Assessing the glomerular function parameters, we observed a significant increase in creatinine clearance (CCr), a marker of estimated glomerular flow rate, only in transgenic rats. However, CCr levels were further increased in the L3292-D group when compared with the L3292-ND group, indicating glomerular dysfunction associated with hyperfiltration in diabetic L3292 rats. Next, we observed a significant increase in glycosuria, renal clearance of glucose, and fractional excretion of glucose as well as proteinuria, urinary protein: creatinine ratio, renal clearance of proteins, and fractional excretion of proteins (FEproteins) in SD-D and L3292-D groups when compared with their respective groups. However, all these parameters, but FEproteins, were more pronounced in the L3292-D group than the L3292-ND group. These data indicate a decrease in tubular reabsorption of glucose, but not of proteins, in diabetic L3292 rats. Interestingly, we observed a significant increase in urinary Gamma-glutamyl transferase, a proximal tubule epithelial injury marker, only in the SD-D group which indicates a protective effect on the tubular structure of transgenic rats. No significant differences were observed between SD-ND and L3292-ND groups in all parameters assessed. Conclusion: Our preliminary data shows that higher systemic levels of Ang-(1-7) promote dual effects on diabetic kidneys. It seems to contribute to developing glomerular dysfunction but protects tubular structures at the early phase of DKD.

Key words: Diabetic kidney disease; renal disease; kidney; hyperglycemia; diabetes mellitus, tubular injury, glycosuria, renin-angiotensin system, angiotensin-(1-7), Sprague Dawley, L3292 rats.